American Petroleum Institute

1220 L Street, Northwest
Washington, DC 20005-4070
Tel: 202-682-8344
Fax: 202-682-8270
E-mail: twerdokl@api.org

Lorraine E. Twerdok, Ph.D., DABT
Manager, Health Sciences

December 20, 2001

Christine T. Whitman, Administrator
1101A
USEPA Headquarters
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Attention: Chemical Right-to-Know

American Petroleum Institute (API)
Petroleum HPV Testing Group

HPV Registration Number

RE: HPV Test Plan and Robust Summaries Submission for the Gasoline Category

Dear Ms. Whitman:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Gasoline Test Plan and Robust Summaries. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substance categories and subsequent test plans. We are therefore submitting the test plan, as well as robust summaries directly to EPA to make available for public comment.

Electronic copies, in .PDF format, of the test plan and robust summaries, are accompanying this letter via email to the EPA HPV robust summary email address (http://www.epa.gov/chemrtk/srbstsum.htm). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have concerning this submission.

Sincerely,

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee
Petroleum HPV Testing Group Technical Committee

Final
12/20/2001
Consortium #

GASOLINE BLENDING STREAMS TEST PLAN

Submitted to the US EPA

by

The American Petroleum Institute
Petroleum HPV Testing Group

Consortium Registration #

GASOLINE BLENDING STREAMS TEST PLAN

Table of Contents

PLAIN LANGUAGE SUMMARY

This test plan focuses on unleaded motor gasoline blending streams. The 87 substances in the Gasoline Blending Streams Test Plan are volatile liquids at standard temperature and pressure and are referred to as low boiling point naphthas. Gasoline-blending naphthas are complex petroleum mixtures consisting of paraffinic hydrocarbons (normal and branched-chain), olefinic hydrocarbons, naphthenic hydrocarbons (cycloparaffins), and aromatic hydrocarbons (mainly alkylbenzenes), with carbon numbers typically ranging from C4 to C12. These four basic chemical classes generally present in all naphthas - **P**araffins, **O**lefins, **N**aphthenes and **A**romatics- are identified by the acronym PONA. The basic strategy of this test plan for characterizing the human health and environmental hazards is to use data from naphthas that are higher in one of these four chemical classes to demonstrate the boundaries of toxicity based on composition for materials in this test plan and to predict the potential hazards of untested naphtha streams.

A substantial body of data has been compiled on representative blending streams and on motor gasoline. These naphthas demonstrate consistently low acute toxicity by oral, dermal and inhalation exposure, are only mildly irritating to the eye, are mild to moderate skin irritants and are not skin sensitizers. Results of repeat dose mammalian studies for naphtha streams enriched in paraffins, olefins and aromatic constituents demonstrate that, when administered dermally, these compounds can be skin irritants with the only systemic effects related to skin damage and accompanying stress; when administered by inhalation, minimal toxic effects are demonstrated with the exception of light hydrocarbon nephropathy in kidneys of male rats, a species and sex specific syndrome not relevant to human health (EPA, 1991). These streams are not genotoxic and do not cause significant reproductive or developmental effects. Extensive testing of gasoline is consistent with results from tests on these streams.

With regard to environmental hazards, the hydrocarbon constituents in gasoline blending-naphthas and gasoline have been demonstrated, by testing and modeling, to be volatile and biodegradable. Although all these petroleum constituents have a low potential for partitioning into water, soil, and sediment, spills or releases from containment, may result in direct impacts to water, soil or sediment quality. Equilibrium solubilities of naphtha constituents are a function of their neat solubilities and mole fractions resulting in consistently low concentrations (ppb to low ppm) in natural waters (surface water and ground water). Nevertheless, where receiving water dilution volumes are low, aquatic toxicity values may be exceeded. The degree of aquatic toxicity (fish, invertebrate and algal) for streams high in paraffins, olefins and aromatics varies within and between streams but generally ranges from approximately 1mg/l to 200 mg/l, quantified as “loading rates”.

Mammalian and environmental data on naphtha blending streams high in naphthenes (cycloparaffins) is more limited than for other naphthas. A Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test (OECD protocol 422) and a biodegradation study (OECD protocol 301F) of a selected high naphthenic blending stream are proposed to complete the gasoline blending streams toxicity profile.

The currently available data and proposed testing outlined in Table 2 combined with chemical characterization will provide sufficient information to predict health and environmental hazards of the 87 materials in the gasoline blending streams category.

GENERAL DESCRIPTION OF HPV SUBSTANCES

The 87 substances in the Gasoline Blending Streams Test Plan, which are volatile liquids at standard temperature and pressure and referred to as low boiling point naphthas, are primarily used to blend unleaded motor gasoline. These naphthas are Class II substances on the Toxic Substances Control Act (TSCA) Chemical Inventory. Class II substances are defined as "Chemical Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials. Appendix I is a complete list of substances included in this Test Plan

The substances in this test plan share many physical properties that make them suitable for gasoline blending but few, if any, could be sold as finished gasoline. There is no significant human exposure to most of these substances, which are blended directly into gasoline and are not present outside the refinery pipelines. However, some naphthas could be used as solvents and the ACC Hydrocarbon Solvents Panel has sponsored several substances that are used for that purpose. Other naphthas from steam-cracking operations, are being sponsored by the ACC Olefins Panel. The Petroleum HPV Testing Group has worked with ACC to ensure that there is no duplication of testing between the three groups.

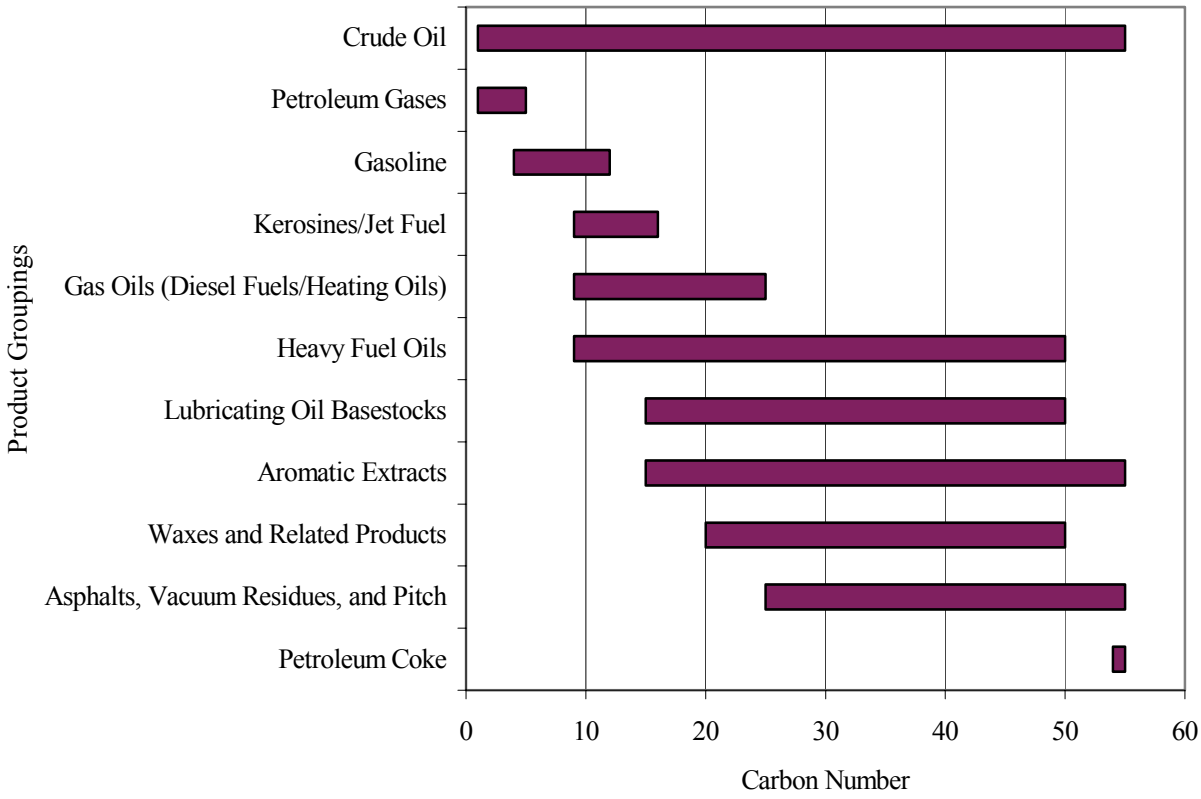
To select test samples to characterize the range of naphtha streams blended into gasoline, the Petroleum HPV Testing Group is using chemical-oriented groupings based on the four primary chemical classes found in naphthas. They are; paraffins, olefins, naphthenes, and aromatics (PONA).

Refining of Gasoline Blending Streams

Gasoline blending streams are refined from petroleum, or crude oil, an extremely complex substance. The hydrocarbon molecules in crude oil may include from one to 50 or more carbon atoms. At room temperature, hydrocarbons containing one to four carbon atoms are gases; those with five to 19 carbon atoms are usually liquids; and those with 40 or more carbon atoms are typically solids. Figure 1 below shows the typical carbon chain lengths found in the proposed HPV

test plans and demonstrates the overlap that occurs.

Figure 1.



Petroleum refining uses distillation as well as chemical treatment, catalysts, and pressure to separate and combine the basic types of hydrocarbon molecules into petroleum "streams" which have the characteristics needed for blending commercial petroleum products. Distillation is not a precise procedure and refining processes vary from refinery to refinery. As a consequence there is not a sharp cut-off between each of the streams that have been separated, and this results in an overlap of substances that occurs in each of the streams. However, streams used in the blending of gasoline must generally fall in a boiling range of –4 to 446^{0}F (–20 to 230^{0}C) and a carbon number distribution of C4-C12.

In addition to primary distillation, numerous refining processes produce the naphthas for blending gasoline. These processes include alkylation, catalytic cracking, catalytic reforming, hydrocracking, hydrodesulfurization, hydrotreating, isomerization, polymerization, sweetening, and thermal cracking. Application of various refining steps is determined by the quality of the initial petroleum crude and product specifications, and produce naphthas with similar carbon numbers and boiling range but with differing molecular compositions. The characteristic chemical composition of naphtha streams is described by PONA classification – the **P**araffinic, **O**lefinic, **N**aphthenic and **A**romatic classes in the stream; within each class, the hydrocarbons also vary in size. All petroleum crude oils contain paraffins, naphthenes and aromatics; olefins are produced during cracking processes. Some refining processes create naphtha that contain predominately one or two of these classes. For example, naphtha from catalytic reforming typically contains high concentrations of aromatics, while alkylation naphtha typically contains no aromatics. Other

refinery processes do not significantly influence the chemical composition of the naphtha. Primary distillation and sweetening would be examples of such processes.

Category Rationale

Gasoline is manufactured to meet property limits, which comply with performance specifications and government regulations, and those property limits, in turn, influence its chemical composition. Specifications limit the boiling range over which naphthas used to blend gasoline can be distilled. Each hydrocarbon boils at a specific temperature and boiling point increases with molecular size. The temperature limits for the gasoline distillation profile excludes smaller hydrocarbons with lower boiling points and larger hydrocarbons with higher boiling points. Figure 2 shows the carbon number distribution of a typical gasoline (C4-C12). Figure 2A illustrates how the cumulative carbon number distribution parallels the distillation profile. As the temperature increases over the gasoline boiling range, molecules with higher carbon numbers are increased in the distillate mix (e.g. a sample collected at 200^0F would contain primarily C4-C6 hydrocarbons, while one collected at 400^0F would also contain C7-C10 hydrocarbons).

Figure 2

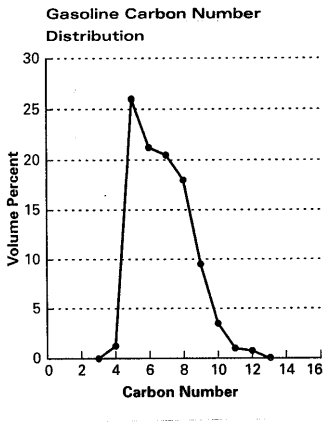
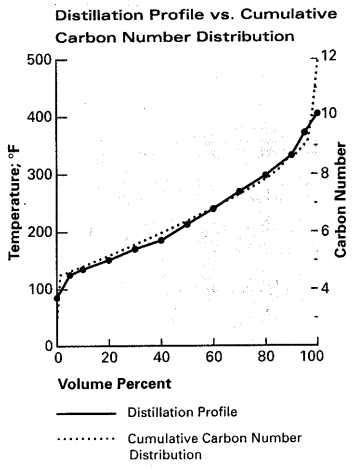


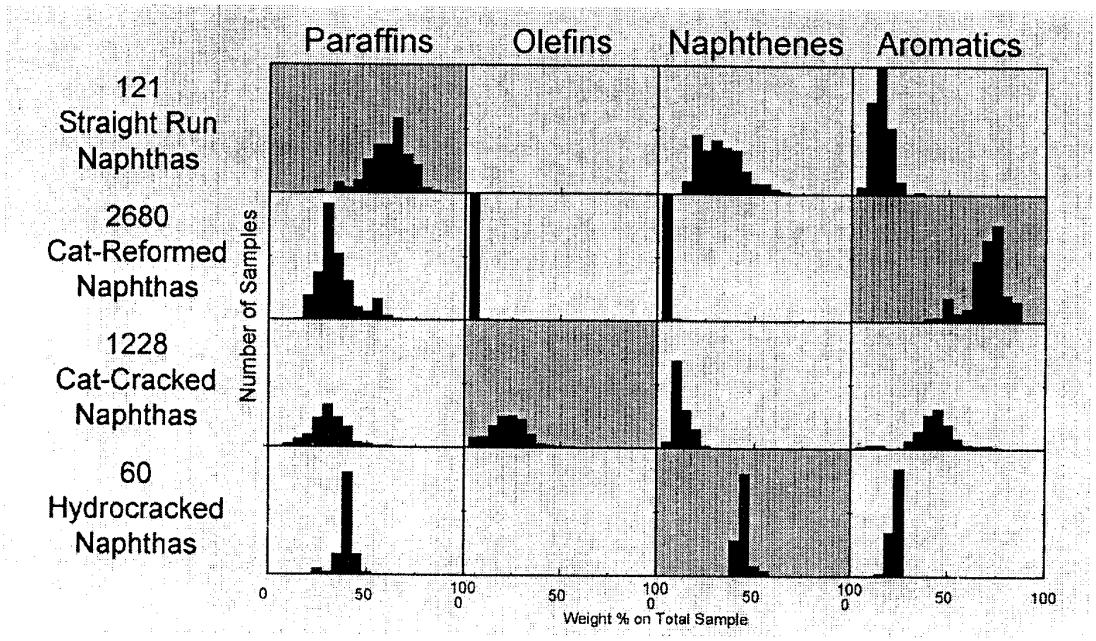
Figure 2A



The hydrocarbons that comprise gasoline and its blending streams – paraffins, olefins, naphthenes (cycloparaffins) and aromatics - share some structural features but differ in the ratio of hydrogen to carbon atoms and how these atoms are arranged.

Figure 3 (below) illustrates distribution of PONA classes for gasoline blending streams from a major refiner: 121 straight run naphtha streams, 2680 catalytic reformed streams, 1228 catalytic cracked streams and 60 hydrocracked streams derived from a wide range of crude oils were analyzed, and the distribution of molecules by weight % for each class are presented. All streams contain paraffins, naphthenes and aromatics in varying concentrations while olefins are present almost exclusively in cracked stocks.

Gasoline Stream Example
Composition Varies Within and Between Groups



These frequency diagrams show quantitatively the spectrum of PONA that can occur in different naphthas. These differences can be exploited to efficiently test the range of potential chemical composition of all 87 substances in this test plan. A key point of this analysis is that even naphthas that have significant levels of one chemical class (i.e. aromatics in catalytic reformed naphtha) usually contain some amount of hydrocarbon from the other chemical classes. It should also be noted that not all of the 87 HPV streams can be classified as either high P, O, N, or A – these hydrocarbon classes may be more evenly represented in the mixture. However, knowledge of the biological activity of representative naphtha streams enriched in an individual PONA class combined with data on the gasoline product make it possible to predict toxicity potential for untested streams with defined PONA characteristics. These data can also be employed internationally to contribute to hazard and risk characterization, preventing unnecessary duplication of testing and reducing animal usage.

PONA CLASSES AND TEST MATERIALS

The Petroleum HPV Testing Group will select four streams to represent the four extremes of hydrocarbon composition. Evaluation of existing data and future testing of these streams will be used to provide relevant information on the 87 HPV substances. Data on at least one naphtha stream from each of the four chemical classes (PONA) will be summarized or obtained through additional testing. In many instances, data on several naphthas in the same chemical category are already available and will be included in the robust summaries. In addition, existing data on

several different samples of gasoline will also be included in the robust summaries. The four representative naphthas are arrayed below within the appropriate PONA category.

Category: HIGH PARAFFINIC
Test Material**:** Naphtha, light alkylate
CAS # 64741-66-8
This test substance is virtually 100% paraffins, by analysis.

Category: HIGH OLEFINIC
Test material: Naphtha, light catalytic cracked
CAS #64741-55-5
This test substance has greater than 40% olefins, by analysis.

Category: HIGH NAPHTHENIC
Test Material: Naphtha, heavy straight-run
CAS # 64741-41-9)
This test substance must be obtained and should contain in the range of 30% naphthenes.
Alternatively, where analytical confirmation confirms similar high percentage of naphthenic content, existing test data for other naphtha streams (i.e., existing data for light straight run naphtha, CAS # 64741-46-4, Concawe sample ID W94/809, 34% naphthenics) are acceptable.

Category: HIGH AROMATIC
Test Material: Naphtha, catalytic reformed
CAS # 68955-35-1
This test substance has greater than 60% aromatics, by analysis.
Alternatively, where analytical confirmation confirms similar high percentage of aromatic content, existing test data for other naphtha streams (i.e., existing data for light catalytic reformed naphtha, CAS # 64741-63-5, Concawe sample W94/812, 63% aromatics) are acceptable.

The naphthas selected for evaluation are used in the blending of gasoline and contain at least as much or substantially more of a given chemical class as is found in gasoline. Thus, to predict the hazard of the contribution from olefins, which occur in average unleaded gasoline at approximately 9%, a light catalytically cracked naphtha containing >40% olefins has been selected. To determine possible effects attributable to aromatic compounds, present in gasoline at 33%, a catalytically reformed naphtha containing >60% aromatics is evaluated. There is a substantial body of data currently available from testing of refinery streams and gasoline samples to address potential toxic effects for many endpoints related to paraffins, olefins and aromatics. As illustrated in Table 1, naphthas enriched in one chemical class also contain components of other classes to contribute to gasoline composition. Since naphthenes (cycloparaffins) are present in gasoline and most finished blending streams at similar concentrations (5-10%), data for a cycloparaffin-rich stream will have to be acquired using an intermediate refinery stream such as a hydrocracked naphtha, (20-26% naphthenes).

Table 1 Contribution of Chemical Classes From Refinery Streams to Gasoline

	Gasoline	Light Alkylate Naphtha.	Light Catalytic Cracked Naphtha	Light Hydrocracked Naphtha	Sweetened Naphtha	Full Range Catalytic Reformed Naphtha
Carbon No.	C4-C12	C5-C10	C5-C10	C4-C9	C4-C10	C5-C12
Boiling range ^{0}C	30-260	90-160	-20 to 190	65-230	39-200	30-220
PONA Classes (Volume %)[a]						
Paraffins	52.8	99.4	30.6	71.3	72.1	32.1
Olefins	9.3	0.0	45.6	0.0	<0.1	0.5
Naphthenics	4.7	0.6	10.4	26.1	20.9	3.7
Aromatics	33.1	0.0	13.1	2.67	6.9	63.3

a- Definitive chemical analysis by Mass Spectroscopy

EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

Results of studies on naphthas high in paraffinic, olefinic and aromatic constituents are summarized in this section. The mammalian toxicology and environmental profiles on these blending streams are supported by comparable test results on gasoline from studies in the US and Europe (see Appendix 3). In addition, a testing program currently in progress mandated by the Clean Air Act 211(b) statute on an EPA designated "industry average" gasoline vapor condensate will provide even more current data on mammalian toxicity of gasoline. Detailed study information is available in the Robust Summaries organized in the IUCLID data set format employed by the European Union (Appendix 5). The currently available data submitted to the HPV program and any additional testing will be developed with the goal of facilitating international harmonization of hazard and risk characterization worldwide. The EU categories for gasoline components, organized by the definitive processing step to produce those components and complementary to the PONA approached employed in this plan are presented in Appendix 4.

Acute Toxicity

(Paraffinic)

Light alkylate naphtha (API 83-19; CAS #64741-66-8; approx 100% paraffinic) is not acutely toxic by the oral (rat > 7000mg/kg), dermal; (rabbit > 2000mg/kg) and inhalation (rat > 5mg/l, 4 hr exposure) routes and is non-irritating to the rabbit eye 24 hrs after exposure. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs.

(Olefinic)

Light catalytically cracked naphtha (API 83-20; CAS #64741-55-5, approx. 46% olefinic) is not acutely toxic by the oral (rat > 5000mg/kg), dermal (rabbit > 3000mg/kg) and inhalation (rat > 5.3mg/l, 4 hr exposure) routes and is not irritating to the rabbit eye 24 hrs after exposure. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs.

(Naphthenic)

Sweetened naphtha (API 81-08, CAS #64741-87-3, approx. 21% naphthenics) is a light straight run naphtha in which a sweetening process has converted mercaptans and removed acidic

impurities. It is not acutely toxic by the oral (rat > 5000mg/kg), dermal (rabbit > 2000mg/kg) and inhalation (rat > 5.2mg/l, 4 hr exposure) routes and is not irritating to the rabbit eye 24 hrs after exposure and only a mild skin irritant in rabbits.

(Aromatic)

Full range, catalytic reformed naphtha (API 83-05, CAS #68955-35-1, approx. 63% aromatics) is not acutely toxic by the oral (rat = 3500-9800mg/kg), dermal (rabbit > 2000mg/kg) and inhalation (rat > 5.22mg/l, 4 hr exposure) routes and is not irritating to the rabbit eye 24 hrs after exposure. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs.

Summary: Results of testing naphtha blending streams for acute toxicity indicate that these materials demonstrate consistently low toxicity by the oral, dermal and inhalation exposure routes, are only mildly irritating to the eyes, are mild to moderate skin irritants and are not skin sensitizers. Acute data for gasoline gave comparable results. Since a heavier stream with a higher naphthenic content will be used for testing in place of sweetened naphtha, acute toxicity information for this stream will be derived as read-across from the existing data. **There is sufficient data to characterize the acute toxicity endpoints of all four categories and no additional testing is necessary**

Repeat Dose Toxicity

(Paraffinic)

Light alkylate naphtha (LAN, CAS #64741-66-8; approx 100% paraffinic) has been tested in the rabbit by dermal exposure, and a vapor distillate fraction has been tested by inhalation in the rat for systemic toxicity and neurotoxicity.

Dermal treatment of New Zealand White rabbits, 3 times/wk for 4 wks. at concentrations of 200, 1000, and 2000mg/kg/day resulted in mild skin irritation at the lowest dose and moderate skin irritation at the mid and high doses in both sexes, in association with granulopoiesis of bone marrow in the highest dose group. Significantly lower body weights were observed in both sexes at 2000mg/kg; organ wt changes included increased adrenal weights in males and decreased ovary weight in females at the highest dose. Adrenal weight changes and granulopoiesis are related to skin irritation induced stress.

Sprague Dawley rats were exposed to a LAN light end distillate at concentrations of 0, 668, 2220, and 6646ppm (2438, 8102 and 24300mg/m^3) 5 days/wk for 13 weeks, according to OECD guideline 413. No test material related mortality or effects on physical signs, body weight. or food consumption, in neurobehavioral tests or neuropathology were observed. Statistically significant increases in kidney weights in high dose males correlated with microscopically observed hyaline droplet formation and degeneration of proximal renal tubules were observed, indicative of light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to humans (EPA, 1991). Increased liver weights in high dose rats of both sexes had no microscopic correlate and appeared reversible after 4 weeks of recovery.

(Olefinic)

Light catalytically cracked naphtha (LCCN, CAS #64741-55-5, approximately 46% olefinic) was tested by inhalation in one 21-day and three 13-week studies. In the 21-day study (15 actual exposures), wholly vaporized LCCN was administered to male Sprague Dawley rats at concentrations of 55, 567, and 3628ppm [200, 2040, and 13060mg/m^3] (Halder et al., 1984). In the three 13 week studies, dosage concentrations were 147 - 2136ppm (530-7690 mg/m^3) partially vaporized LCCN to rats and mice (Dalbey and Feuston, 1996); 1500 – 4500ppm (5474-16423 mg/m^3) wholly vaporized LCCN to rats (API, 1987); and 750 – 7500ppm (2336-23364

mg/m^3) light ends distillate to rats (Lapin et al., 2001). In all studies, over a wide range of doses and exposure durations of 3-13 weeks, the most significant treatment related effect was an increase in male kidney weights with increased incidence of hyaline droplets and degeneration of proximal renal tubules indicative of light hydrocarbon nephropathy, at the highest dose. In the 1987 API study, increases in liver weights in both sexes was accompanied by centrilobular heptocellular hypertrophy at the highest dose (4500ppm) in males only. Results of the Lapin et al, 2001 study which employed OECD guideline 413 (Combined subchronic toxicity and neurotoxicity screening), demonstrated that exposure to LCCN vapor did not induce neurobehavioral effects or neuropathologic damage to brain, spinal cord or peripheral nerves. Hyperplasia and hypertrophy of nasal epithelium observed at the high dose was not evident after 4 weeks of recovery.

(Naphthenic) -

The only repeat dose study performed in this category was a 2 year mouse skin painting study to evaluate the dermal carcinogenesis of sweetened naphtha (CAS #64741-87-3). Sweetened naphtha caused skin irritation, but did not induce cancer or other indications of target organ toxicity.

(Aromatic)

Repeat dose studies have been performed on three materials in this stream category: Full range catalytic reformed naphtha (FR-CRN, CAS #68955-35-1) – 28 day dermal study in rabbits (API, 1986), and a 13 week inhalation study in rats with a partially vaporized sample (Dalbey and Feuston, 1996); Light catalytic reformed naphtha (LCRN, CAS #64741-63-5)- 21 day inhalation study in rats with a fully vaporized sample (Halder et al., 1984), and a 13 week inhalation study in rats with light end distillate sample (Schreiner et al., 2000); Heavy catalytic reformed naphtha (HCRN, CAS #64741-68-0) – 21 day inhalation study in rats with a fully vaporized sample (Halder, 1984).

FR-CRN: In the 28 day dermal study FR-CRN was applied to the shaved backs of New Zealand White rabbits, 3 times a week for 4 weeks at doses of 200, 1000 and 2000mg/kg/day. Three males (2 high dose, 1 mid dose) died. Test material was a moderate-severe skin irritant. Inhibition of body weight and weight loss occurred at 2000mg/kg. Histopathologic examination revealed slight-moderate proliferative and inflammatory changes in skin at the highest dose concurrent with granulopoiesis of bone marrow, attributed to stress and other factors associated with skin irritation. No other significant findings were reported.
For the 13-week inhalation study, Sprague Dawley rats were exposed to FR-CRN, partially vaporized (30-40%) to produce a vapor with composition similar to human exposure, at concentrations of 0, 96, 464, and 1894ppm (0, 410, 1970, 8050mg/m^3), 5 days/wk. No significant biological effects were observed with the exception of higher liver and kidney weight in high dose males. No treatment related abnormalities were seen in any tissue examined histologically.

LCRN and HCRN: In the 21 day inhalation studies, male Sprague Dawley rats were exposed to a light reformate naphtha (31% aromatics) and a heavy reformate naphtha (93% aromatics) at concentrations of 0, 544, 1591, and 5522ppm (0, 2000, 5850 and 20300mg/m3) LCRN or 0, 215, 587, and 2132ppm (1030, 2810, and 10200mg/m3) HCRN for 15 actual exposures. LCRN induced small concentration related increases in necrosis of renal tubules and an increase in incidence and severity of hyaline droplets, typical of light hydrocarbon nephropathy. Exposure to HCRN did not cause adverse effects in the kidney but lung irritation was apparent. In the 13 week study, male and female Sprague Dawley rats were exposed to a light vapor fraction of LCRN at concentrations of 750, 2500, and 7500ppm (2775, 9250 and 27,750mg/m^3). No test material related mortality or effects on physical signs, body weight, food consumption or clinical

chemistry were observed. In males exposed to 7500ppm, a statistically significant decrease in white blood cell and lymphocyte counts, and a decrease in spleen weight were observed at terminal sacrifice, but were not present in animals after a 4-week recovery period. Statistically significant increase in kidney weight relative to body weight in high dose males correlated with microscopically observed light hydrocarbon nephropathy. The only effect on neurobehavioral parameters was significantly higher motor activity in high dose males after the 4-week recovery period without exposure to LCRN distillate, but there was no evidence of hyperactivity or abnormal behavior from the functional observational battery and no microscopic changes in neural tissue (Schreiner et al., 2000).

Summary: Results of repeat-dose dermal studies of blending streams and gasoline indicate that these materials are generally skin irritants with the only systemic effects related to skin damage and related stress at high doses. Inhalation studies with naphtha streams demonstrated minimal toxic effects with the exception of light hydrocarbon nephropathy in the kidneys of male rats at the highest dose. Subsequent research with naphthas and gasoline demonstrated light hydrocarbon nephropathy to be a species and sex specific syndrome, not relevant to human health (EPA, 1991). This nephrotoxic activity appears attributable to the alkane constituents**.** Streams that contain a higher proportionate content of aromatic components including benzene and toluene, such as heavy catalytic cracked naphtha, do not produce this syndrome. Streams tested for neurotoxicity did not induce any significant neurobehavioral or neuropathologic effects. There is sufficient data from repeat-dose studies to characterize the paraffinic, olefinic and aromatic blending steams, supported by comparable data for gasoline product (Appendix 3). Only the naphthenic stream category has insufficient repeat dose data. **A repeat dose inhalation study in rats (OECD protocol 422) using a naphtha stream high in naphthenic content (e.g. heavy straight run naphtha or heavy hydrocracked naphtha) is proposed to complete this toxicity endpoint.**

In Vitro Genetic Toxicity

(Paraffinic)

Light alkylate naphtha diluted in acetone, has been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay and did not induce mutagenicity with or without metabolic activation from rat liver homogenate.

(Olefinic)

Three samples of light catalytically cracked naphtha have been tested in the mouse lymphoma (L5178Y TK+/-) forward mutation assay and did not induce mutagenicity with or without metabolic activation from rat liver homogenate, with the exception of equivocal results with metabolic activation for one sample with a higher ratio of aromatic constituents (20.3%) compared to 10-13% aromatics in other samples.

A sister chromatid exchange assay in Chinese hamster ovary cells with and without metabolic activation with LCCN produced negative results without, but equivocal results with activation.

(Naphthenic)

Sweetened naphtha, diluted in ethanol, tested in the mouse lymphoma (L5178Y TK+/-) forward mutation assay, did not induce mutagenicity with or without metabolic activation from rat liver homogenate.

(Aromatic)

Light catalytic reformed naphtha (42% aromatics) did not induce mutagenic events in the mouse lymphoma (L5178Y TK+/-) forward mutation assay with or without metabolic activation from rat liver homogenate. Full range, catalytic reformed naphtha (63% aromatics) did not induce mutagenicity without metabolic activation but did induce a dose responsive positive mutagenic effect with metabolic activation. Heavy catalytic reformed naphtha (90% aromatics) was also positive with metabolic activation and induced equivocal results without activation.

Summary: Gasoline blending streams and gasoline show little if any mutagenic activity in *in vitro* test systems. Where activity is present, it occurs with metabolic activation and can be correlated with a higher ratio of aromatics in the test sample (60-90%) than is characteristic of the distribution of aromatics in gasoline, the product (approx. 30% aromatics). *In vitro* genetic toxicity potential of a high naphthenic stream selected for testing will be estimated from chemical composition and by read-across from sweetened naphtha and gasoline test results. **There is sufficient data to characterize the *in vitro* genetic toxicology endpoint for all four PONA categories and no additional testing is necessary.**

In Vivo Genetic Toxicity

(Paraffinic)

Light alkylate naphtha was tested in a rat chromosome aberration assay at doses of 0.3, 1.0, and 3.0g/kg in corn oil, administered intraperitoneally in a single dose. Animals were sacrificed at 6, 24 and 48 hrs post dose. Deaths occurred in both male and females in the highest dose group and a 10% body weight loss was observed in surviving rats of both sexes. No chromosome aberrations, rearrangements, or cell cycle disruption were observed in any dose group.

(Olefinic)

Samples of light catalytically cracked naphtha were tested in both *in vivo* mouse sister chromatid exchange (SCE) and rat chromosome aberration assays. In the SCE assay, mice were given a single intraperitoneal dose at concentrations of 0.2, 1.2 and 2.4g/kg in corn oil and bone marrow lymphocytes were evaluated for evidence of exchange of genetic segments between sister strands, indicative of DNA perturbation; LCCN induced SCE in this assay. In one rat chromosome aberration assay, when animals were treated with a single intraperitoneal dose at concentrations of 0.3, 1.0, and 3.0g/kg and sacrificed at 6, 24, and 48 hrs post-dose, LCCN did not induce chromosome aberrations or cell cycle disruption. In a separate chromosome aberration assay, rats were exposed by inhalation to 63, 297, and 2046ppm (230, 1084, and 7467mg/m^3) for 5 days. LCCN did not induce chromosome aberrations or inhibit normal cell cycle kinetics. Although the SCE assay demonstrated interaction of LCCN and DNA, it is not definitive for clastogenic activity since no genetic material is unbalanced or lost. Negative results in two assays, which visualize actual cytogenetic damage demonstrate that LCCN is not a clastogenic material.

(Naphthenic)

Sweetened naphtha was tested in a rat chromosome aberration assay by inhalation at concentrations of 65, 300, and 2050ppm (215, 993 and 6788mg/m^3) for 5 days. Animals were sacrificed 6 hrs after the final dose. Sweetened naphtha did not induce chromosome aberrations or disruption to cell cycle kinetics.

(Aromatic)

Full range, catalytic reformed naphtha (FR-CRN), light catalytic reformed naphtha (LCRN) and heavy catalytic reformed naphtha (HCRN) were tested in rat chromosome aberrations assays

with a single intraperitoneal injection of test material in corn oil at concentrations of approximately 0.3, 1.0 and 2.5-3.0g/kg. Rats were killed at 6, 24 and 48hrs post-dose to evaluate all stages of cell cycle in bone marrow lymphocytes. None of these materials induced chromosome aberrations or disruption of cell cycle kinetics in these assays.

Summary: Gasoline blending streams and gasoline are not clastogenic. Gasoline also did not induce heritable effects in male mice reflected in post-implantation deaths or reduced fertility (Appendix 3). Potential for cytogenetic damage for the high naphthenic stream selected for repeat dose testing will be estimated from existing data on sweetened naphtha (21% naphthenic) and on gasoline (approximately 5% naphthenic) as well the absence of activity shown by other naphtha streams. **There is sufficient data to characterize the *in vivo* genetic toxicology endpoint and no additional testing is necessary.**

Reproductive And Developmental Toxicity

(Paraffinic)

Light alkylate naphtha: A light vapor fraction of LAN administered to rats by inhalation at target concentrations of 0, 500, 12500 and 25000mg/m^3 (0, 137, 3425, and 6850ppm) according to OECD protocol 421: Reproductive/developmental toxicity screening test, did not induce reproductive or systemic effects in treated male and female rats. All pregnant females had comparable delivery data and pups in all groups showed comparable birth weights, weight gain, and viability at postnatal day 4. No histopathological changes were seen at necropsy for adults or offspring, and reproductive organs of adult animals were normal histologically. NOAEL for all endpoints= 25000mg/m^3 (Bui et al., 1998).

(Olefinic)

Light catalytically cracked naphtha was tested for reproductive and developmental effects in two assays. In a developmental toxicity screen, presumed pregnant Sprague Dawley rats were exposed to 0, 2150, and 7660mg/m^3 (0, 597, and 2128ppm) partially vaporized LCCN from day 0-19 of gestation. Females were sacrificed on day 20. Number of resorptions was increased at the highest dose level but no other treatment related changes were observed (Dalbey et al., 1996). A distillate of LCCN administered to rats by inhalation at target concentrations of 0, 2700, 9000, and 27000mg/m^3 (0, 750, 2500, and 7500ppm) according to OECD protocol 421, did not affect reproductive performance, delivery data, or live pups/litter. Offspring showed comparable body weights, weight gain, and viability index at postnatal day 4. Parental male rats had increased kidney weights and relative liver weights at the highest dose, and high dose females had increased spleen weights. Reproductive organs and nasal turbinates from high dose and control animals were examined by a pathologist, and no histological changes were observed in tissue from treated rats. NOAEL parental toxicity = 9000mg/m^3; NOAEL reproductive performance/ developmental toxicity = 27000mg/m^3 (Schreiner et al., 1999).

(Napththenic)

No reproductive/developmental studies are available for this category.

(Aromatic)

Full range, catalytic reformed naphtha: A developmental toxicity screen was performed with partially vaporized (30-40%) FR CRN administered by inhalation to presumed pregnant Sprague Dawley rats at concentrations of 0, 2160, and 7800mg/m^3 (0, 508, and 1835ppm) on gestation days 6-19. Animals were sacrificed on day 20 of gestation. Maternal body weights, serum chemistry and organ weights were unaffected. No adverse effects were observed on fetal

parameters at sacrifice (viability, fetal body weight, external development) or subsequent skeletal and visceral examinations (Dalbey and Feuston, 1996).
A distillate of light catalytic reformed naphtha administered to Sprague Dawley male and female rats by inhalation at target concentrations of 0, 2775, 9250, and 27750mg/m^3 (0, 750, 2500, and 7500ppm) according to OECD protocol 421, did not affect reproductive performance, delivery data or live pups/litter. Offspring showed comparable body weights, weight gain and viability index at postnatal day 4. Parental systemic effects observed at the highest dose were slightly reduced body weights for males, increased kidney to body weight and liver to body weight ratios. Reproductive organs and nasal turbinates from high dose animals and controls were examined by a pathologist and no histological changes were observed in tissue from treated rats. NOAEL parental toxicity = 9250mg/m^3; NOAEL reproductive/developmental toxicity = 27750mg/m^3 (Schreiner et al, 2000).

Summary: There is sufficient data to characterize developmental and reproductive toxicity of paraffinic, olefinic and aromatic blending streams. The absence of naphtha-induced significant toxicity for these endpoints is supported by comparable data on gasoline (Appendix 3). However, there is no test data for any stream representative of the high naphthenic category; therefore **screening for developmental and reproductive effects in rats as part of OECD protocol 422, using a stream high in naphthenic content (e.g. heavy straight run naphtha or heavy hydrocracked naphtha) is proposed to complete this toxicity endpoint.**

EVALUATION OF EXISTING PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA

The physicochemical endpoints for the EPA HPV chemical program include melting point, boiling point, vapor pressure, water solubility, and octanol/water partition coefficient (Kow). Environmental fate endpoints include biodegradation, photodegradation, hydrolysis, and fugacity. Because the HPV substances covered under the testing plan are mixtures of differing compositions, it is not possible to measure or calculate a single numerical value for some of the physicochemical properties. For example, a product that is a mixture of chemicals does not have a melting point, but rather a melting point range. Melting point, boiling point and vapor pressure range will be reported because these substances are complex mixtures. Values for PC properties will be represented as a range of values according to the product's component composition. Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured representative data will be identified and a technical discussion provided where appropriate. The EPIWIN© computer model, as discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. " is used to calculate physical/chemical properties of representative PONA constituents for selected naphtha streams. The hydrocarbon components in these selected naphtha streams have been identified by detailed hydrocarbon analysis using gas chromatography coupled with flame ionization detection and/or mass spectrometry (GC/FID, GC/MS). Log P_{ow}, atmospheric oxidation half-lives and environmental media partitioning were calculated for these individual hydrocarbon constituents identified by GC/FID or GC/MS in specific naphthas, and the range of these properties are summarized.

Summary: Where measured data does not exist and is impractical to develop, calculated physicochemical and environmental data for selected constituents of gasoline blending streams have been developed using the EPIWIN© computer model**.**

Partition Coefficient: calculated log P_{ow} at 25^0C

(Paraffinic)	Light alkalyte naphtha	3.11-4.54 (C5-C9)
(Olefinic)	Light catalytic cracked naphtha	2.13-4.54 (C5-C9)
(Naphthenic)	Lt. straight run naphtha (high naphthenic)	2.73-4.85 (C5-C9)
	Lt. straight run naphtha (moderate naphthenic)	2.13-4.76 (C5-C9)
	Lt. straight run naphtha (low naphthenic)	2.13-4.00 (C5-C7)
(Aromatic)	Light catalytic reformed naphtha	2.13-4.54 (C5-C9)
Gasoline		2.13-4.50 (C5-C8)

Summary: Range of partition coefficients for gasoline and blending streams is 2.13-4.85.

Water Solubility: determined from preparations of water accommodated fractions
Calculated and measured water solubilities differ for individual components of complex petroleum substances. At any particular loading rate, aqueous concentrations of each component is a function of relative volume of aqueous and petroleum phases, partition coefficient between phases, amount of component present and the maximum water solubility of each component.

(Paraffinic): Light alkylate naphtha
freshwater 1.6ppm equilibrium at 24 hrs.
saltwater 0.9ppm equilibrium at 12 hrs.
based on chromatographic analysis of combined concentrations of alkyl butanes, alkyl pentanes and dimethyl hexane comprising 68% of test substance.

(Olefinic): Light catalytic cracked naphtha
freshwater 4.6ppm equilibrium at 24 hrs.
saltwater 4.3ppm equilibrium at 12 hrs.
based on analysis of benzene, toluene, ethylbenzene, o-xylene, p-xylene comprising 13% of test substance.

(Naphthenic): Light straight run naphtha (high naphthenic)
freshwater 5.7-7.9ppm equilibrium at 19 hrs. based on chromatographic analysis of toluene, ethyl benzene and xylene comprising approx. 13% of test substance.
Light straight run naphtha (low naphthenic) freshwater 4.9ppm as benzene equilibrium at 24 hrs. based on chromatographic analysis of benzene, ethyl benzene, toluene and xylenes.

(Aromatic): Light catalytic reformed naphtha
freshwater 13.7ppm equilibrium at 24 hrs.
saltwater 14.0ppm equilibrium at 24 hrs.
based on total combined concentrations of pentane, 2-methylpentane, benzene, toluene, ethylbenzene, and xylenes comprising 50% of test substance

(Gasoline):
freshwater 3.1, 3.1, <6.9E-3, 0.92ppm as benzene, toluene, ethyl benzene and xylene respectively

Summary: Solubility in fresh and salt water ranged from 1- 14ppm ranked in order of greatest solubility as LCRN>LSRN>LCCN>gasoline>LAN. Although none of the naphtha streams are appreciably water soluble, streams higher in aromatics and naphthenics demonstrate greater

solubility than other streams or gasoline. There is sufficient data on all four PONA categories for this endpoint. **No additional testing is necessary.**

Environmental Fate Data

Environmental fate endpoints include biodegradation, photodegradation, hydrolysis, and fugacity. Biodegradation data, available for several representative naphthas in this category, show that these products can exhibit a moderate to rapid rate of biodegradation. For the photodegradation endpoint, data is calculated. Products in this category are not subject to hydrolysis at measurable rates, therefore, hydrolysis is not a relevant endpoint for these products. Calculated environmental partitioning behavior (fugacity modeling) for selected constituents of the naphtha streams indicate that these chemicals will partition largely to the air, and therefore their fate in air is of environmental interest.

Photodegradation: The direct aqueous photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation. Only light energy at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment, although absorption is not always sufficient for a chemical to undergo photochemical degradation. In general, most products in the Gasoline Naphtha category do not contain component molecules that will undergo direct photolysis. Saturated hydrocarbons (paraffins and naphthenics), olefins with one double bond, and single ring aromatics, which constitute the majority of these components, do not absorb appreciable light energy above 290 nm. Therefore, this fate process will not contribute to a measurable degradative removal of chemical components in this category from the environment.

Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Indirect photolysis can be estimated using models accepted by the US EPA and other authorities. An estimation method accepted by the US EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Hydrocarbon constituents of Gasoline Naphtha Streams, readily volatilize to air. In air, chemicals may undergo reaction with photosensitized oxygen in the form of hydroxyl radicals (OH^-). The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows), used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances), calculates a chemical half-life based on an overall OH^- reaction rate constant, a 12-hr day, and a given OH^- concentration. This AOPWIN calculation will be performed for those hydrocarbon constituents detected in representative naphtha streams for each of the PONA groupings.

Summary: Insufficient data are available to characterize the atmospheric oxidation potential of chemical components found in products in this category. Therefore, representative components for this category will be identified and their AOP values calculated.
AOPWIN version 1.89 calculates atmospheric oxidation half-lives of hydrocarbons in contact with hydroxyl radicals in the trophosphere, under the influence of sunlight and in contact with O_3, based on a 12-hour day at 25^0C.

(Paraffinic): Light alkylate naphtha, calculated for C5-C9 components
½ life range: 1.074 days (2,3,5 trimethylhexane) to 15.985 days (isopentane)

(Olefinic): Light catalytic cracked naphtha, calculated for C5-C9 components
½ life range for constituents due to OH reaction: 2.5 hrs (2-methyl-1-butene) to 15.985 days (isopentane)

½ life range for olefinic constituents (30% of stream composition): 38.378 min (1-methyl cyclopentene) to 22.950 hrs. (C5 olefins)

(Naphthenic): Light straight run naphtha (high naphthenic), calculated for C5-C9 components
½ life range: 0.902 days (toluene) to 2.047 days (m-xylene)
Light straight run naphtha (moderate naphthenic, 19.8%), calculated for C5-C9 components
½ life range: 0.789 days (m-xylene) to 15.985 days (isopentane)
Light straight run naphtha (low naphthenic), calculated for C5-C9 components
½ life range: 1.262 days (isopentane) to 15.985 days (cyclohexane)

(Aromatic): Light catalytic reformed naphtha, calculated for C5-C8 components
½ life range: 1.498 days (2,3 dimethyl pentane) to 15.985 days (isopentane).

(Gasoline): calculated for C5-C8 components
½ life range: 0.789 days (m-xylene) to 15.985 days (isopentane)

Summary: Calculated atmospheric half-lives for naphtha blending streams and gasoline under conditions of 12 hours of sunlight daily, ranged from a minimum of 38.4 min (1-methylcyclopentene in light catalytic cracked naphtha) to approximately 16 days (isopentane or cyclohexane). This modeling was based on detailed hydrocarbon analyses of each stream constituents and the known half-lives of these constituents. Because naphthas are composed of the same groups of hydrocarbons in varying concentrations, it can be concluded that the gasoline blending streams from all 4 PONA categories degrade in sunlight at a rate of one half the overall content within 16 days. **No additional modeling is necessary**

Stability in Water:

Summary: Hydrolysis is unlikely for gasoline and blending streams (C4-C12). Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkylhalides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the naphtha category are hydrocarbons, which are not included in these chemical groups, and they are not subject to hydrolysis reactions with water. **No additional testing or modeling is necessary.**

Chemical Transport and Distribution in the Environment (Fugacity Modeling):

Equilibrium models are used to calculate chemical fugacity that can provide information on where a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. Fugacity data can only be calculated. A widely used fugacity model is EQC (Equilibrium Criterion) model. In its guidance document for HPV data development, the US EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. Level I is a steady state, equilibrium model that utilizes the input of basic physicochemical parameters including molecular weight, vapor pressure, and water solubility. Distribution is calculated as percent of chemical partitioned to the 6 environmental compartments (air, soil, water, biota, suspended sediment and sediment) within a unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition in the environment. Values represent the calculated range of distribution to environmental media of C5-C9 hydrocarbon components found in each stream.

(Paraffinic): Light alkylate naphtha – Mobility in aquatic and terrestrial environment is low due to low water solubility and high vapor pressure. Components partition primarily into air: Air: 99.4-100%; soil 0.01-0.27%, water 0.001-0.01%.

(Olefinics): Light catalytic cracked naphtha – Partitions into air >99% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

(Naphthenics): Light straight run naphtha (High naphthenic) – Partitions into air >97% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%
Light straight run naphtha (Low naphthenic) – Partitions rapidly into air for all components:. Air: 98.9-99.98%; soil 0.01-0.11; water: 0.01-1.0%

(Aromatics): Light catalytic reformed naphtha – Partitions into air >99% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

(Gasoline): Partitions into air >97% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

Summary: Fugacity modeling for those constituents in gasoline blending streams and gasoline indicate that, at steady-state, these petroleum mixtures components partition >97% to air where hydrocarbons are rapidly oxidized by OH radicals. Partitioning into soil or water does not exceed 1.2% or 2.7%, respectively. Partitioning to sediment or suspended sediment is minimal. These data are adequate to define environmental distribution of naphtha streams and gasoline. **No additional modeling is necessary for this endpoint.**

Biodegradation: Analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Selected data for products in this category show that they have the potential to biodegrade to a high extent. These data are based on results of carbon dioxide evolution tests for three products; one that is composed primarily of isoparaffinic hydrocarbons, a second that consisted of iso-paraffinic, olefinic, naphthenic and aromatic hydrocarbons, and a third product composed of linear paraffins, iso-paraffins and aromatic hydrocarbons. The procedure used consists of a closed system, which is recommended when assessing the biodegradability of poorly water soluble, volatile materials like those in this category.
These naphtha streams typically contain several different isomers from the PONA hydrocarbon classes mentioned above. This variety of chemical structure can impede achieving a potential maximum extent of biodegradation within a standard testing period because microbial adaptation to a series of differing isomers and chemical classes is likely to occur with numerous stepwise biodegradation lag phases. This can result in a lag period between chemical classes before a maximum degradation rate is once again achieved with the next class. Typically, these data will not clearly exhibit the occurrence of these stepwise events because of the varied metabolic potentials in a mixed bacterial inoculum. As a consequence, the evaluation of data from standard tests performed with these complex products can lead to an underestimation of biodegradation rate. Therefore an acclimation step was employed as reported in the selected data in order to optimize enzymatic activity in the microbial inoculum by pre-adapting the inoculum individually to each of the naphtha product. Following a 14-day acclimation period, ultimate biodegradability as a measure of CO2 evolution was determined in test systems containing the pre-adapted inoculum and fresh naphtha substrate. These data suggest that products in this category can demonstrate relatively high extents of biodegradability and that they are not expected to persist in the environment.

(Paraffinic): Light alkylate naphtha was tested using a mixed adapted inoculum of domestic activated sludge and soil for 56 days. LAN achieved 42% biodegradation by day 28, slight increase to 48% by day 42, and a return to 40% by day 56, indicating inherent biodegradability.

(Olefinic): Light catalytically cracked naphtha was tested using a mixed adapted inoculum of domestic activated sludge and soil for 56 days. LCCN achieved 75% inherent biodegradation by day 28, which increased slightly to 79% by day 56, indicating inherent biodegradability.

(Napththenic): No biodegradability data is available on any naphthenic sample.

(Aromatic): Light catalytic reformed naphtha was tested using a mixed adapted inoculum of domestic activated sludge and soil for 56 days. LCRN achieved 96% biodegradation by day 28, and maintained a level rate to day 56.

Summary: Biodegradation tests of gasoline blending streams high in paraffins, olefins and aromatics demonstrate inherent biodegradability of 40-96% depending on the stream. Results of these studies are adequate to characterize the P, O, and A streams. The profile is incomplete without biodegradation data on a naphthenic enriched sample. **Testing is proposed for a selected high naphthenic sample using OECD protocol 301F.**

EVALUATION OF EXISTING ECOTOXICITY DATA AND PROPOSED TESTING

The HPV Chemical Test Program includes acute toxicity to a freshwater fish and invertebrate, and toxicity to a freshwater alga. The products in the Gasoline Naphtha Category are expected to produce a similar range of toxicity for these three endpoints based on results of comparable studies using standard test methods and exposure solution preparation procedures since the aquatic toxicity data for selected products within this category can be predicted based on carbon number range and constituent composition of those products. (See Appendix 3, discussion of aquatic toxicity)

Aquatic Toxicity

The following information summarizes reliable representative aquatic toxicity data of selected naphtha streams prepared as WAFs. Additionally, calculated values for fish and invertebrate aquatic toxicity are reported for a Full Range Catalytic Reformed Naphtha (CAS # 68955-35-1, API sample 83-05, high aromatic naphtha). This stream is expected to have a greater aromatic distribution than the light catalytic reformate naphtha (LCRN, CAS # 64741-63-5) for which reliable ecotoxicity data have been summarized. In order to evaluate the impact of a higher percentage of aromatics hydrocarbons, ecotoxicity was estimated (Appendix 3, Calculation Of Acute Toxicity From Composition) since the hydrocarbon composition and percentage distribution were available for this sample. Supporting these estimated values is a comparison to ecotoxicity test data for a LCRN sample developed by Concawe.

(Paraffinic): Light alkylate naphtha, tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 8.2mg/L (95% C.I. 5.2-9.7mg/L) nominal loading rate in fathead minnow and a 48 hr. EL_{50} = 32mg/L (95% C.I. 18-140mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 45mg/L (95% C.I. 18-70mg/L) in *Selenastrum caprocornutum*.

(Olefinic): Light catalytically cracked naphtha tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 46 mg/L (95% C.I. 37-74mg/L) nominal loading rate in fathead minnow

and a 48 hr. EL_{50} = 18mg/L (95% C.I. 13-25mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 64mg/L (95% C.I. 44-111mg/L) in *Selenastrum caprocornutum*.

(Naphthenic): Ecotoxicity results for two light straight run naphtha (low and high naphthenic content) have been evaluated. Robust summaries for a low naphthenic, Light straight run naphtha (approximately 19.8% naphthenic) tested as a water-accommodated fraction (WAF) have been prepared. Light straight run naphtha (approximately 19.8% naphthenic) tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 15mg/L (95% C.I. 6.3-25mg/L) nominal loading rate in fathead minnow and a 48 hr. EL_{50} = 18mg/L (95% C.I. 12-24mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 6.4mg/L (95% C.I. 5.7-7.1mg/L) in *Selenastrum caprocornutum*.

High naphthenic, Light straight run naphtha (Concawe sample W94/809, approximately 34% naphthenic) tested as a water accommodated fraction (WAF), test data reported based on review of gasoline product dossier (Concawe,, Acute, Aquatic Toxicity of Gasolines, report no. 96/57). WAFs of high naphthenic LSRN induced a 96 hr LL50 = 18 mg/L (95% C.I. 15-20 mg/L) based on nominal loading rate in rainbow trout and a 48 hr. EL50 = 4.5 mg/L, nominal loading rate in Daphnia magna. The algal 72 hr EL50 = 3.6 mg/L (95% C.I. 1.7-6.2 mg/L) in Selenastrum capricornutum.

(Aromatic): Light catalytic reformed naphtha tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 34mg/L (95% C.I. 25-50mg/L) nominal loading rate in fathead minnow and a 48 hr. EL_{50} = 10mg/L (95% C.I. 6-12mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 8.5mg/L (95% C.I. 7.3-9.8mg/L) in *Selenastrum caprocornutum*.

High Aromatic (Reformate): Calculated toxicity using hydrocarbon block method and published values:
Full Range Catalytic Reformed Naphtha (CAS # 68955-35-1, API sample 83-05, high aromatic naphtha) Daphnia Acute calculated 48 hr EL50 loading rate =0.9 mg/L; Fish Acute calculated 96 hr LL50 loading rate =2.09 mg/L.
Light Catalytic Reformed Naphtha (CAS # 64741-63-5, Concawe sample W94/812), high aromatic naphtha) Daphnia Acute tested 48 hr EL50 loading rate =8.4 mg/L; Fish Acute tested 96 hr LL50 loading rate =12 mg/L. (Concawe, Acute, Aquatic Toxicity of Gasolines, report no. 96/57.) An analysis of the calculated and reported ecotoxicity for high aromatic reformate naphtha streams having similar composition indicate ecotoxicity between 1 to 10 mg/L WAF loading. The lower measured toxicity of LCRN can likely be attributed to decreased aqueous hydrocarbon concentration resulting from partitioning to vapor headspace, adsorption, and degradation as compared to the more conservative calculated values.

Summary: Aquatic toxicity data is adequate for naphtha streams high in paraffins, olefins, naphthenics and aromatics. Levels of toxicity to different aquatic organisms (the freshwater fish, fathead minnow, aquatic invertebrate, *Daphnia magna*, and alga) varied for each stream and between streams. In general, light alkylate naphtha was the most toxic to fathead minnow, and light straight run naphtha showed greatest toxicity to algae. Sufficient data of good quality were identified to accurately characterize the three aquatic toxicity endpoints in the HPV program for this category. In general, products in this Category have the potential to be moderately toxic to aquatic organisms. **Therefore, no further aquatic testing is proposed.**

Terrestrial Toxicity

Summary: Gasoline blending streams have been demonstrated to be volatile and biodegradable. **No testing is proposed for this endpoint.**

Conclusions and Test Proposal

There is sufficient and adequate data to assess the mammalian toxicity and ecotoxicity of naphtha streams high in paraffinic, olefinic, naphthenic and aromatic constituents; data well supported by toxicity studies on the gasoline product (Appendix 3). Modeling for physical properties and environmental endpoints has been completed for all classes of naphthas.

TABLE 4. MATRIX OF AVAILABLE ADEQUATE DATA AND PROPOSED TESTING FOR THE PRIMARY TEST MATERIALS

	P	O	N	A	
	Naphtha, light alkylate 64741-66-8	Naphtha, light catalytic cracked 64741-55-5	Naphtha, heavy straight-run 64741-78-2	Naphtha, catalytic reformed 68955-35-1	Gasoline
Melting Point	N/A	N/A	N/A	N/A	N/A
Boiling Point	Adequate	Adequate	Adequate	Adequate	Adequate
Vapor Pressure	Adequate	Adequate	Adequate	Adequate	Adequate
Partition Coefficient	TD	TD	TD	TD	TD
Water Solubility	Model/TD	Model/TD	Model/TD	Model/TD	Model/TD
Photodegradation	TD	TD	TD	TD	TD
Stability in Water	Model	Model	Model	Model	Model
Transport and Distribution	Model	Model	Model	Model	Model
Biodegradation	Adequate	Adequate	**Test**	Adequate	C
Acute Toxicity to Fish	Adequate	Adequate	Adequate	Adequate	Adequate
Acute Toxicity to Aquatic Invertebrates	Adequate	Adequate	Adequate	Adequate	Adequate
Toxicity to Algae	Adequate	Adequate	Adequate	Adequate	Adequate
Acute Toxicity	Adequate	Adequate	C	Adequate	Adequate
Repeated Dose	Adequate	Adequate	**Test**	Adequate	Adequate
Genotoxicity, in vitro	Adequate	Adequate	C	Adequate	Adequate
Genotoxicity, in vivo	Adequate	Adequate	C	Adequate	Adequate
Repro/Developmental	Adequate	Adequate	**Test**	Adequate	Adequate

Adequate	Indicates adequate existing data.
Test	Indicates proposed testing
Model	Indicates data will be obtained with EPA approved models
C	Indicates category read-across from existing or proposed test data
TD	Indicates technical discussion to define endpoint
N/A	Indicates that evaluation of endpoint is Not Applicable due to physical-chemical state or route of administration.

There is limited data available on naphtha streams high in naphthenes (cycloparaffins). Therefore this study plan proposes a Combined Repeated Dose Toxicity Study with the Reproductive/ Developmental Toxicity Screening Test (OECD protocol 422) and a biodegradation study (OECD protocol 301F) using a selected naphthenic-rich stream, to complete the hazard profile for gasoline blending streams.

REFERENCES

Mammalian Toxicity

Bui, Q.Q., Burnett, D.M., Breglia, R.J., Koschier, F.J., Lapadula, E.S., Podhasky, P.I., Schreiner, C.A., White, R.D., Dalbey, W.E., and Feuston, M.H. 1998. Toxicity evaluation of petroleum blending streams: Reproductive and developmental effects of a distillate from light alkylate naphtha. *J. Toxicol. Environ. Health* 53: 121-133.

Chevron Products Co. 1996. Motor Gasoline Technical Review (FTR-1) San Francisco, CA.

Dalbey, W., and Feuston, M. 1996. Partially vaporized full range catalytic reformed naphtha: Subchronic and developmental toxicity studies in rats. *Inhalation Toxicol*. 8: 271-284.

Dalbey, W.E., Feuston, M.H., Yang, J.J., Kommineni, C.V., and Roy, T.A. 1996. Light catalytically cracked naphtha: Subchronic and developmental toxicity screen in rats. J. *Toxicol. Environ. Health* 47: 77-91.

Halder, C.A., Warne, T.M., and Hatoum, N.S. 1984. Renal toxicity of gasoline and related petroleum naphthas in male rats. Chapter VI in Renal Effects of Petroleum Hydrocarbons, Mehlman et al., Eds. Princeton Scientific Publishers, Princeton, NJ pp. 73-88.

Halder, C.A., Van Gorp, G.S., Hatoum, N.S., and Warne, T.M. 1986. Gasoline vapor exposures Part 1. Characterization of workplace exposures. *Am. Ind. Hyg. Assocs. J*.; 47: 164-172.

Kuna, R.A., and Ulrich, C.E. 1984. Subchronic inhalation toxicity of two motor fuels. *J. Am. Coll. Toxicol*. 3: 217-230.

Lapin, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Podhasky, P., Lapadula, E., Roth, R., Schreiner, C., White, R., Mandella, R., and Hoffman. 2001. Toxicity Evaluation of Petroleum Blending Streams: Inhalation Subchronic Toxicity/Neurotoxicity Study of Light Catalytic Cracked Naphtha Distillate in Rats. *Internat'l J. of Toxicology: in press*

MacFarland, H.N., Ulrich, C.E., Holdsworth, C.E., Kitchen, D.N., Halliwell, W.H., Blum, S.C. A chronic inhalation study with unleaded gasoline vapor. *J. Am. Coll. Toxicol*. 1984; 3: 231-248.

McKee, R.H., Trinmer, G.W., Whitman, F.T., Nessel, C.S., Mackerer, C.R., Hagemann, R., Priston, R.A., Riley, A.J., Cruzan, G., Simpson, B.J., Urbanus, J.H. 2000. Assessment in rats of the reproductive toxicity of gasoline from a gasoline vapor recovery unit. *Reproductive Toxicol.* 14: 337-353.

Roberts, L., White, R., Bui, Q., Daughtrey, W., Koschier, F., Rodney, S., Schreiner, C., Steup, D., Breglia, R., Rhoden., Schroeder., and Newton, P. 2001. Developmental toxicity evaluation of unleaded gasoline vapor in the rat. *Reproductive Toxicol.* 15:

Schreiner, C., Lapadula, E., Breglia, R., Bui, Q., Burnett, D., Koschier, F., Podhasky, P., White, R., Mandella, R., and Hoffman, G.: 1998. Toxicity Evaluations of Petroleum Blending Streams: Inhalation Subchronic Toxicity/ Neurotoxicity Study of a Light Alkylate Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 55: 277-296.

Schreiner, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Lapadula, E., Podhasky, P., White, R., and Schroeder, R.E.: 1999. Toxicity Evaluation of Petroleum Blending Streams: Reproductive and Developmental Effects of Light Catalytic Cracked Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 58:101 –118

Schreiner, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Podhasky, P., White, R., Hoffman. G., and Schroeder, R.E.: 2000. Toxicity Evaluation of Petroleum Blending Streams: Reproductive and Developmental Effects of Light Catalytic Reformed Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 60:101 – 116.

Schreiner, C., Breglia, R., Bui, Q., Burnett, D., Koschier, F., Lapadula, E., Podhasky, P., White, R., Hoffman, G. and Mandella, R. 2000. Toxicity Evaluations of Petroleum Blending Streams: Inhalation Subchronic Toxicity/Neurotoxicity Study of a Light Catalytic Reformed Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 60: 489 – 513

U.S. Environmental Protection Agency. Alpha 2 microglobulin: association with chemically induced renal toxicity and neoplasia in the male rat. 1991. In Risk Assessment Forum. US Government Printing Office, Washington, DC: EPA:85

U.S. Environmental Protection Agency Fuels and Fuels Additives Registration Regulations; Final Rule pursuant to the Clean Air Act 211(b), 211(e). 40 CFR Part 79, FR59, No. 122: 33042-33142 (27 June 1994). Washington, DC:EPA. 1994

U.S. Environmental Protection Agency. Fuels and Fuels Additives Registration Regulations; Final Revised 211(b) Tier2 Testing Requirements. Correspondence to Director of Health and Environmental Sciences Dept., American Petroleum Institute. November 2, 1998.

Ecotoxicity

Cline, P.V. et al. 1991. Partitioning of aromatic constituents into water from gasoline and other complex solvent mixtures. *Environ Sci Technol 25: 914-920*

CONCAWE. 1996. Environmental risk assessment of petroleum substances: the hydrocarbon block method. Report No. 96/52. Brussels: CONCAWE

Deneer, J.W. et al. 1988. The joint toxicity to *Daphnia magna* of industrial organic chemicals at low concentrations. *Aquatic Toxicology* 12: 33-38).

Di Toro, D.M., J.A. McGrath, and D.J. Hansen. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environmental Toxicology and Chemistry* 19: 1951-1970.

Hermens, J.L.M. et al. 1985. Quantitative structure-activity relationships in aquatic toxicity studies of chemicals and complex mixtures. *Environ Toxicol Chem* 4: 273-279.

McCarty, L.S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. *Environmental Science and Technology* 27:1719-1728.

McCarty, L.S. et al. 1991. Interpreting aquatic toxicity QSARs: the significance of toxic body residues at the pharmacologic endpoint. In: Hermens, J.L.M. and Opperhuizen, A. (Eds). QSAR in environmental toxicology. Volume IV, p. 515-525. Amsterdam: Elsevier

Peterson, D.R. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere* 29,(12)*: 2493-2506*

Solano-Serena, F., R. Marchal, D. Blanchet, and J-P. Vandecasteele. 1998. Intrinsic Capacities of Soil Microflorae for Gasoline Degradation. *Biodegradation* 9:319-326.

Solano-Serena, F., R. Marchal, M. Ropars, J.-M. Lebeault, and J.-P. Vandecasteele. 1999. Biodegradation of Gasoline: Kinetics, Mass Balance and Fate of Individual Hydrocarbons. *J. Appl. Microbiol*. 96:1006-1016.)

Van Wezel, A.P. and A. Opperhuizen. 1995. Narcosis Due to Environmental Pollutants in Aquatic Organisms: Residue-Based Toxicity, Mechanisms, and Membrane Burdens. *Critical Reviews in Toxicology* 25:255-279.

Verbruggen et al. 2000. Polyacrylate-Coated SPME Fibers as a Tool To Simulate Body Residues and Target Concentrations of Complex Organic Mixtures for Estimation of Baseline Toxicity**.** *Environ. Sci. Technol*. 34: 324-331

Appendix 1

Gasoline Category Constituents by CAS

The CAS numbers and descriptions for refinery streams were developed in response to Section 8(b) of the Toxic Substances Control Act which required identification and registration with the Environmental Protection Agency, before July 1979, of each "chemical substance" being manufactured, processed, imported or distributed in commerce. Due to analytical limitations and known variability in stream composition, identification of every specific individual molecular compound in every refinery stream process under all processing conditions was impossible. American Petroleum Institute (API) recommended to EPA a list of generic names for refinery streams covering all known processes used by refiners. A definition of each stream was included and published with CAS numbers by EPA as "Addendum I, Generic Terms Covering Petroleum Refinery Process Streams" In these definitions, process history, specifically the final process step, and not chemical composition, was one of the primary criteria to differentiate streams and assign CAS numbers. As a result, streams with the same or substantially similar compositions may have different CAS numbers if they originate in different process units. Thus, the 87 naphtha CAS numbers in the gasoline blending stream category do not mean there are large compositional differences between streams. It simply reflects the fact that these streams, comprised of the same basic hydrocarbons in varying concentrations, are produced by a large number of process units within a refinery. Organization of these naphtha streams by composition, based on Paraffin, Olefin, Naphthene and Aromatic content, regardless of CAS number, is the most practical way of evaluating for biological effects.

CAS Number	Substance
008006619	Gasoline, natural
008030306	Naphtha from natural gas
008032324	Ligroine
008052413	Stoddard solvent
064741419	Naphtha (petroleum), heavy straight-run
064741420	Naphtha (petroleum), full-range straight-run
064741464	Naphtha (petroleum), light straight-run
064741475	Natural gas condensates (petroleum)
064741486	Natural gas (petroleum), raw liquid mix
064741544	Naphtha (petroleum), heavy catalytic cracked
064741555	Naphtha (petroleum), light catalytic cracked
064741635	Naphtha (petroleum), light catalytic reformed
064741646	Naphtha (petroleum), full-range alkylate
064741657	Naphtha (petroleum), heavy alkylate
064741668	Naphtha (petroleum), light alkylate
064741680	Naphtha (petroleum), heavy catalytic reformed
064741691	Naphtha (petroleum), light hydrocracked
064741704	Naphtha (petroleum), isomerization
064741726	Polymerization naphtha, intermediate C6-C12
064741748	Naphtha (petroleum), light thermal cracked
064741782	Naphtha (petroleum), heavy hydrocracked
064741839	Naphtha (petroleum), heavy thermal cracked
064741840	Naphtha (petroleum), solvent-refined light
064741873	Naphtha (petroleum), sweetened
064741920	Naphtha (petroleum), solvent-refined heavy
064741997	Extracts (petroleum), light naphtha solvent
064742229	Naphtha (petroleum), chemically neutralized heavy
064742230	Naphtha (petroleum), chemically neutralized light
064742489	Naphtha (petroleum), hydrotreated heavy
064742490	Naphtha (petroleum), hydrotreated light
064742730	Naphtha (petroleum), hydrodesulfurized light
064742821	Naphtha (petroleum), hydrodesulfurized heavy

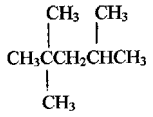
064742898	Solvent naphtha (petroleum), light aliph.
064742956	Solvent naphtha (petroleum), light aromatic
067891796	Distillates (petroleum), heavy arom.
067891809	Distillates (petroleum), light arom.
068333299	Residues (petroleum), light naphtha solvent extracts
068410059	Distillates (petroleum), straight-run light
068410719	Raffinates (petroleum), cat. reformer ethylene glycol-water countercurrent exts.
068410968	Distillates (petroleum), hydrotreated middle, intermediate boiling
068410979	Distillates (petroleum), light distillate hydrotreating process, low-boiling
068410980	Distillates (petroleum), hydrotreated heavy naphtha, deisohexanizer overheads
068425310	Gasoline (natural gas), natural
068475796	Distillates (petroleum), catalytic reformed depentanizer
068476437	Hydrocarbons, C4-C6, C5-rich
068476460	Hydrocarbons, C3-C11 catalytic cracker distillates
068476506	Hydrocarbons, C>=5, C5-C6-rich
060476551	Hydrocarbons, C5-rich
068476562	Hydrocarbons, cyclic C5 and C6
068477349	Distillates (petroleum), C3-C5, 2-methyl-2-butene-rich
068477634	Extracts (petroleum), reformer recycle
068477894	Distillates (petroleum), depentanizer overheads
068478126	Residues (petroleum), butane splitter bottoms
068478159	Residues (petroleum), C6-C8, catalytic reformer
068478160	Residual oils (petroleum), deisobutanizer tower
068513020	Naphtha (petroleum), full-range coker
068513031	Naphtha (petroleum) light catalytic reformed, arom.-free
068513633	Distillates (petroleum), catalytic reformed straight-run naphtha overheads
068514158	Gasoline, vapor recovery
068514385	Hydrocarbons, C4-C10 Unsaturated
068514794	Petroleum products, hydrofiner-powerformer reformats
068526523	Alkenes, C6-rich
068526556	Alkenes, C9-rich
068527219	Clay treated naphtha, full range, C4-C11
068527264	Naphtha (petroleum) light steam-cracked, debenzenized, C4-C12
068527275	Naphtha (petroleum, full-range alkylate), butane contg.
068551166	Alkanes, C9-C11-iso
068551177	Alkanes, C10-C13-iso
068602799	Distillates (petroleum), benzene unit hydrotreater dipentanizer overheads
068603010	Distillates (petroleum), thermal cracked naphtha and gas oil, C5-dimer-contg
068603087	Naphtha (petroleum), arom.-contg.
068606111	Gasoline, straight-run, topping-plant
068783119	Polymerization naphtha, light C5-C11
068783120	Naphtha (petroleum), unsweetened
068783664	Naphtha (petroleum), light, sweetened
068919153	Hydrocarbons, C6-C12, benzene-recovery
068919379	Naphtha (petroleum), full-range reformed
068919391	Natural gas condensates
068920069	Hydrocarbons, C7-9
068921084	Distillates (petroleum), light straight run gasoline fractionation stabilizer overheads
068921095	Distillates (petroleum), naphtha unifiner stripper
068955293	Distillate (petroleum), light thermal cracked, debutanized arom.
068955351	Naphtha (petroleum), catalytic reformed
070024929	Alkanes, C7-C8-iso
070693060	Aromatic hydrocarbons, C9-C11
070955087	Alkanes, C4-C6
092045584	Isomerization naphtha

APPENDIX 2

Petroleum Chemistry and Refining

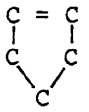
The hydrocarbons that comprise gasoline and its blending streams - paraffins, olefins, naphthenes (cycloparaffins) and aromatics – share some structural features but differ in the ratio of hydrogen to carbon atoms and how those atoms are arranged.

Paraffins: C_nH_{2n+2} where n= number of carbon atoms.
Carbons are joined by single bonds (e.g. butane, $CH_3CH_2CH_2CH_3$). Paraffins with 4 or more C atoms may have 2 or more structural arrangements or structural isomers for example: normal octane, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or isooctane

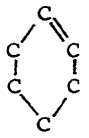


Olefins: C_nC_{2n} are similar to paraffins but have 2 fewer hydrogen atoms and contain at least one double bond (e.g. 2-butene, $CH_3CH=CHCH_3$). Olefins with 4 or more carbons can exist as structural isomers. Cyclic olefins are present in cracked products and are found mostly in motor gasoline, for example:

C_5H_8 Cyclopentene

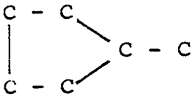


C_6H_{10} Cyclohexene

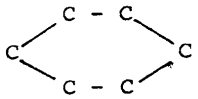


Naphthenes: Cycloparaffins in gasoline have 5 or 6 carbon atoms arranged in a ring and belong to either a cyclopentane or cyclohexane series, for example:

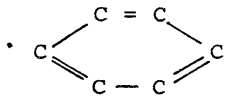
C_6H_{12} Methylcyclopentane



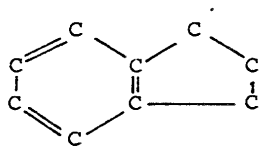
C_6H_{12} Cyclohexane



Aromatics: Some carbon atoms are arranged in a ring joined by aromatic bonds. for example:



benzene, C_6H_6 . In polycyclic aromatics, some carbons are shared by 2 or more rings, for example, indane, C_9H_{10}



A Short Course in Gasoline Refining

Petroleum crude oils range in appearance from thin and light-colored to as thick and black as melted tar. Thin, light crudes contain more natural gasoline and lower sulfur and nitrogen content, making them easier to refine to high value products like gasoline; heavier thick crudes require more rigorous refining processes, more energy, and greater cost to produce high value products. All crudes are composed of hydrocarbons of the paraffinic, naphthenic and aromatic classes; olefins are produced during refining. Each class contains a broad range of molecular weights with a broad range of boiling points.

Distillation is the basic step in producing gasoline and other products from crude oil. Crude oil is heated and product is obtained by condensing the vapor that boils off over a specified temperature range at atmospheric pressure. In a distillation column, the vapor with the lowest boiling hydrocarbons (propane and butane) rises to the top. Straight run gasoline, kerosene and diesel fuel are drawn off at successively lower positions in the columns at higher boiling temperature. Hydrocarbons with boiling points higher than diesel fuel can't be vaporized; they remain as liquids in the bottom of the column (atmospheric bottoms). Application of a vacuum to the distillation column improves the high value product yield.

Cracking is a process used to produce higher quality products, including gasoline, from the atmospheric bottoms. Hydrocarbons with higher boiling points can be broken down (Cracked) by breaking carbon to carbon bonds into lower boiling hydrocarbons by subjecting them to very high temperature (Thermal cracking). Olefins are produced through the cracking process. When a catalyst is employed to supplement heating, this Catalytic cracking produces a gasoline of higher quality than thermal cracking. The catalyst speeds up or facilitates the chemical reaction without undergoing permanent chemical damage itself. Fluid catalytic cracking (FCC) is a standard method in modern refineries in which the solid catalyst id fluidized to allow circulation from the reaction section of the cracker to the regeneration section and back again.

Hydrocracking employs a catalyst in a hydrogen atmosphere to break down hydrocarbons resistant to catalytic cracking alone, and is used primarily to produce diesel fuel.

Reforming literally reorganizes the petroleum feed, converting straight chain paraffins into more complex aromatic hydrocarbons that contribute to octane level.

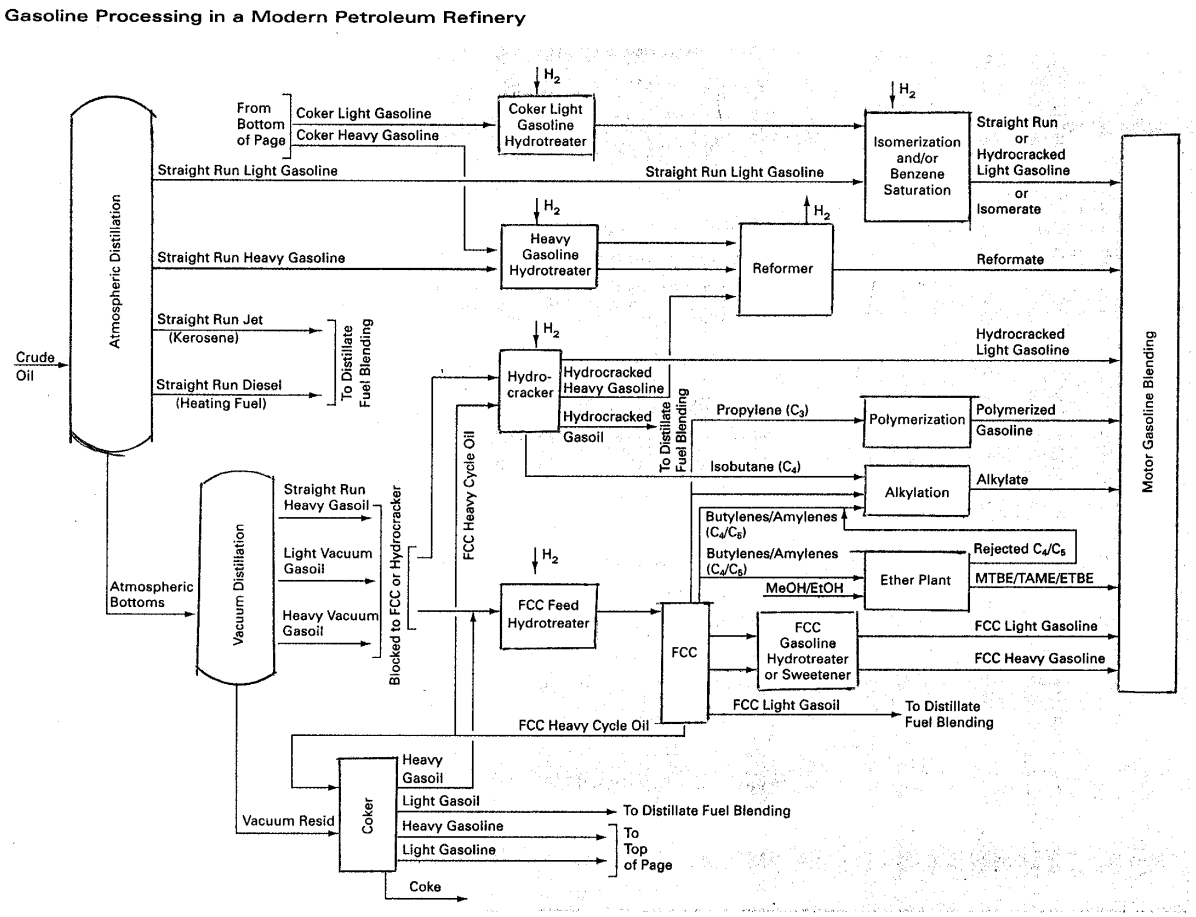
Octane quality defines the ability of gasoline to burn smoothly and uniformly without explosion (knock) in the engine. Octane rating is determined by measuring fuel performance in an engine against that of iso-octane (100 octane rating). The higher the octane rating the more efficiently the fuel burns, resulting in more power per gallon. Aromatics and olefins are high octane hydrocarbons but their content is gasoline has been reduced due to environmental concerns, so other methods of improving octane are employed.

Alkylation combines small, gaseous hydrocarbons with boiling points too low for use in gasoline to form liquid hydrocarbons with higher boiling points. Alkylation is a key process in producing reformulated gasolines because the content of other classes of high octane hydrocarbons – olefins and aromatics- are limited by regulation.

Other conversion processes include polymerization that combines small olefins (C3, propylene) into larger olefins (C6, C9, C12) and isomerization which converts straight chain paraffins (C5, C6) into their branched isomers to improve octane value.

Hydrotreating identifies a range of processes that use hydrogen with catalyst to remove impurities from a refinery stream to improve the product. Mild, selective hydrotreating is used to remove highly reactive olefins, while heavy hydrotreating converts aromatic to naphthenes. Desulfurization, a form of hydrotreating, removes sulfur to comply with lower sulfur limits in reformulated gasolines, and to protect the catalyst that can be deactivated by excess sulfur in the stream.

The schematic layout of a modern refinery is shown in figure below.



Crude oil is fed to the distillation column where straight run light and heavy gasoline, jet and diesel are separated at atmospheric pressure. Straight run jet and diesel fuels are acceptable as is; straight run gasolines must be further processed before blending into gasoline product. Straight run light gasoline may be isomerized to increase octane, or hydrotreated to convert benzene to cyclohexane so that the final gasoline blend meets a benzene specification limit. Straight run heavy gasoline is hydrotreated to remove sulfur and then reformed to improve octane and generate hydrogen for the hydrotreaters.

The bottoms from the atmospheric column are vacuum distilled to produce gasoils for the FCC or hydrocracker feed. Gasoils are hydrotreated to reduce sulfur and nitrogen to levels that do not interfere with FCC cracking. The FCC product must also be sweetened to convert sulfur compounds (mercaptans) to more innocuous compounds to eliminate odor and instability in the gasoline blend.

The vacuum residuum is sent to a resid conversion unit (e.g. resid cracker, solvent extraction unit or coker) to produce more transportation fuel. These resid-derived streams require further processing before they can be blended into light fuels like gasoline or diesel.

APPENDIX 3

Gasoline Mammalian Toxicity

Acute toxicity

Gasoline (API PS-6) is similar to its component blending streams. It is not acutely toxic by the oral (rat > 18.75ml/kg [14g/kg]), dermal (rabbit > 5ml/kg [3.9g/kg]) routes and is not irritating to the rabbit eye 24 hrs after exposure. It is a mild skin irritant in rabbits and is not a skin sensitizer in guinea pigs.

Repeat Dose Toxicity

Thirteen week inhalation toxicity studies were performed with wholly vaporized leaded and unleaded gasoline at concentrations of 0, 100 and 400ppm, or 0, 400, 1500ppm (1493, 5597 mg/m^3) respectively, in Sprague Dawley rats and squirrel monkeys (API, 1976, Kuna and Ulrich, 1984). Light hydrocarbon nephropathy was induced in kidneys of male rats exposed to leaded or unleaded gasoline but not in kidneys of squirrel monkeys. In rats, slight increases in platelet counts and liver weights of high dose males occurred with exposure to both gasolines, with increases in tissue and urinary lead levels for animals given leaded gasoline. Monkeys showed a small increase in respiratory rate with exposure to the highest dose of unleaded gasoline, a dose that was 4 times higher than that of leaded gasoline. A two year inhalation carcinogenesis bioassay was performed with wholly vaporized unleaded gasoline at actual concentrations of 0, 67, 292 and 2056ppm (250, 1089, 7672mg/m^3) administered to rats and mice (API, 1983, McFarland et al, 1984). Mortality rates were unaffected. Rats and mice in the highest dose group had lower body weights throughout the study. Kidney weights of male rats were elevated accompanied by light hydrocarbon nephropathy at interim sacrifices and dose related incidences of kidney tumor at terminal sacrifice. These kidney lesions have been determined to be species and sex specific and not relevant to humans (EPA, 1991). In mice, liver tumors were present in high dose females.

A testing program currently in progress under Clean Air Act 211(b) includes a 13 week rat inhalation study of “industry average” gasoline vapor at concentrations of 2000, 10000 and 20000 mg/m3 (650, 3250 and 6500ppm) which also includes neurotoxicity, immunotoxicity, and cytogenetic endpoints. The vapor condensate of an EPA designated “industry average” gasoline was distilled by a method acceptable to EPA that produce a light end vapor similar in composition to vehicle exposure emissions. This test material induced light hydrocarbon nephropathy with minimal other systemic effects but did not cause neurobehavioral or neuropathologic effects and did not cause immunotoxic responses in spleen cells.

In Vitro Genetic Toxicology

Unleaded gasoline samples, diluted in dimethyl sulfoxide, tested in the Ames *Salmonella* microbial mutation assay and in the mouse lymphoma (L5178Y TK+/ -) forward mutation assay did not induce mutagenic events with or without metabolic activation in either test system. Gasoline was also negative in an Unscheduled DNA synthesis assay in rat hepatocytes. In the CAA 211(b) test program, “industry average” gasoline vapor is being tested in the Ames *Salmonella* assay with and without metabolic activation

In Vivo Genetic Toxicology

Unleaded Gasoline has been tested for induction of chromosome aberrations in rat bone marrow cells, and for transmittable genetic effects in the mouse dominant lethal assay. In the rat chromosome assay, animals were given a single intraperitoneal dose of 18.5, 62.0, and 185mg/rat (0.024, 0.08 and 0.24ml/rat) or one dose each day for 5 days at concentrations of 7.7, 23.1, and 77mg/rat (0.01, 0.03, and 0.10ml/rat/day). Gasoline did not induce chromosome

aberrations or disruption of cell cycle kinetics in either regime at any dose level. In the CAA 211(b) test program currently in progress, "industry average" gasoline vapor is being evaluated for sister chromatid exchange in peripheral blood, and chromosome aberrations in bone marrow of rats exposed in a 13 week inhalation study.

In the dominant lethal assay, gasoline was administered by inhalation to male mice at concentrations of 400 and 1600ppm (1493 and 5970mg/m^3), 6hr/day, 5 days/wk for 8 weeks over the entire mouse spermatogenic cycle. At termination of exposure, males were mated with untreated females; females were then sacrificed 14 days after mating (approx. 2/3rd through pregnancy) and uterine contents evaluated. Gasoline exposure of male mice did not cause any significant reduction in fertility index, did not affect the number of total implants or number of dead implants/pregnant female.

Reproductive and Developmental Toxicity

Unleaded gasoline and gasoline vapor have been tested for developmental and reproductive effects. Pregnant Sprague Dawley rats were exposed by inhalation to unleaded gasoline vapor at concentrations of 0, 1493, and 5970mg/m^3 (0, 400 and 1600ppm) from day 6-15 of gestation; caesarean sections were performed on day 20. There were no treatment related effects on any reproductive parameter (pregnancy ratio, live litters, implantation sites, litters with resorptions, dead fetuses, litter size, fetal weights), or fetal soft tissue or skeletal examination (API, 1978). An unleaded gasoline vapor condensate (10.4% by volume of starting gasoline) was also evaluated for developmental toxicity in pregnant Sprague Dawley rats by inhalation at concentrations of 0. 2653, 7960, and 23900mg/m^3 (0, 1000, 3000, and 9000ppm) from day 6-19 of gestation according to US EPA TSCA test guideline 798-4350. No maternal toxicity was observed. At caesarean section on day 20 of gestation, no treatment related effects were observed on any reproductive parameter (pregnancy ratio, live litters, implantation sites, litters with resorptions, dead fetuses, litter size, fetal weights) or fetal malformations or variations. NOAEL for maternal and developmental toxicity = 23900mg/m^3. (Roberts et al, 2001).

Vapor recovery gasoline was evaluated by inhalation for reproductive toxicity in a 2 generation reproductive toxicity screen in Sprague Dawley rats at concentrations of 0, 5000, 1000, and 20000mg/m^3 (0, 1850, 3700 and 7400ppm) in accordance with OECD protocol 416 and US EPA OPPTS 870.3800 draft guideline for reproduction and fertility effects (1994). There were no treatment related systemic in parental females and only the species and sex specific hyaline droplet nephropathy was observed in kidneys of male rats of both generations. No reproductive parameters were affected and there were no deleterious effects on offspring survival and growth. Sperm count and quality were comparable in all dose groups. NOAEL reproductive toxicity = 20000mg/m^3 (McKee et al, 2000). In the Clean Air Act 211(b) test program, "industry average" gasoline vapor is being evaluated in both a developmental toxicity assay and a 2-generation reproduction assay (in progress).

Gasoline Environmental Toxicity (Experimental data only)

Aquatic Toxicity-Mode of Action

The aquatic toxicity data for selected products within this category can be predicted based on carbon number range and constituent composition of those products. This is because the constituent chemicals of those products are neutral organic hydrocarbons whose toxic mode of action is non-polar narcosis. The toxic mechanism of short-term toxicity for these chemicals is disruption of biological membrane function (van Wezel and Opperhuizen, 1995), and the differences between toxicities (i.e., LC/LL50, EC/EL50) can be explained by the differences between the target tissue-partitioning behavior of the individual chemicals (Verbruggen et al., 2000). The existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of between approximately 2-8 mmol/kg fish (wet weight) (McCarty and Mackay, 1993; McCarty et al., 1991). When normalized to

lipid content the CBR is approximately 50 µmol/g of lipid for most organisms (Di Toro et al., 2000). Products in this category are multi-constituent hydrocarbons containing various combinations of isomeric structures (i.e., n-paraffinic, isoparaffinic, cycloparaffinic, olefinic and aromatic) and with carbon (C) numbers ranging primarily between C4 to C12.

Multi-constituent hydrocarbon solvent products with a range of carbon numbers and water solubility as those in this category are expected to exhibit lower toxicity compared to the most toxic constituent alone. This occurs because the aqueous concentration of the constituent is a function of the partitioning of the constituents between the bulk hydrocarbon and water. Within the carbon number range of products in this category, a C9 hydrocarbon alone would be expected to exhibit the greatest toxicity based on the relationship of Kow with aquatic toxicity. However, products in this category are not composed of a single chemical and because two different products with a similar carbon number range can contain varying proportions of those carbon numbers, it is possible that different toxicities are expressed for the same organism. Thus, two products representing low or high carbon number ranges in this category can show different toxicities. Therefore, characterizing the fish, daphnid, and algal toxicity of this category using values from the low and high carbon number ranges is supported.

The endpoint values for the three trophic levels reflect the loading rates of the test substance added to exposure solutions prepared as water accommodated fractions (WAF) in closed test systems. The WAF method is described in the relevant Robust Summaries provided with this test plan. This method is the appropriate procedure for products in this category because these products are multi-constituent hydrocarbons whose constituent hydrocarbons vary in water solubility. The dissolution thermodynamics of a multi-constituent hydrocarbon in an aqueous medium prevent the possibility of achieving consistent proportional concentrations of the constituent hydrocarbons at various test substance loading rates. For this reason:

- exposure solutions are not prepared from dilutions of a stock solution (the relative proportion of hydrocarbon constituents in the dilutions would not accurately reflect the relative concentration of those constituent chemicals in individually prepared, successively lower exposure solutions of the test material);

- separate exposure solutions are prepared at each exposure loading for products that are multi-constituent hydrocarbons; and

- results for multi-constituent hydrocarbons are expressed as lethal loadings (LL) rather than lethal concentrations (LC) as is possible for single, water-soluble chemicals.

CALCULATION OF ACUTE TOXICITY FROM COMPOSITION

There are two situations when it may be necessary to estimate the toxicity of a petroleum substance viz., to validate test results and to predict toxicity when data are lacking. This approach requires that the chemical composition of the petroleum substance should be known. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below). As previously described, the quantity of any particular component of a petroleum substance detected in the water phase is related to the loading rate. Theoretically, using closed test systems brought to equilibrium, simple equilibrium partitioning and mass balance calculations may be used to estimate the concentration of each hydrocarbon constituent in water. The hydrocarbon/water partition coefficient (Kp) for each of the components is an essential part of the calculation. The details of this calculation approach have been published (Peterson, D.R., 1994). Further simplification is

obtained by combining the concentration calculations for isomers of particular hydrocarbon species (e.g. iso-hexanes), since all of the isomers have essentially the same values of log Kow and Kp. This procedure is the equivalent of the "hydrocarbon block method" used in the risk assessment of petroleum substances (CONCAWE, 1996; Hermens, J.L.M. et al., 1985). Experimental Kp values (Peterson, D.R., 1994; Cline, P.V. et al., 1991) simply related to Kow, for individual hydrocarbons are available in the published literature. In order to calculate the joint toxic action of a mixture of hydrocarbons dissolved in water, the concentrations cannot be added directly. Since each component will have a different toxicity, the concentration of each component must be scaled to its toxicity. This is done by division of the concentration by the toxicity (by the LL5O in the case of acute toxicity). The resulting values express the concentrations in equivalent "toxic units." Thus, the sum of TUs for the components of a mixture will equal one at the LL50 of the mixture. Considerable experimental support for this conceptual framework has been developed, which confirms that mixtures of substances exerting toxicity via a common mechanism, are additive and further, that hydrocarbons act through a common mechanism of non-polar narcosis (Hermens, J.L.M. et al, 1985; Deneer, J.W. et al., 1988). Toxicity QSARs may be used to provide LL50 estimates for hydrocarbons or blocks where acute toxicity data are not available, since these are well established for hydrocarbons (details are included in the EU Technical Guidance Document (TGD) which recommends procedures for risk assessment). Furthermore, the use of QSAR allows for extrapolation of LL50 values to hydrocarbons or blocks that are beyond the solubility ‘cut-off' and have no measured LC50 value. This provides a conservative approach for assessing the partial contribution of hydrocarbons or blocks that are individually not expected to exert toxicity.

In summary, given the compositional analysis (together with consideration of the variability of composition of the particular petroleum substance), acute toxicity can be calculated. This toxicity calculation is conservative in that it assumes that each component is maximally dissolved (completely equilibrated with undissolved phase and there is no competition for solubility between similar hydrocarbons) and that there are no losses from solution (due to adsorption to surfaces, absorption to test organisms or volatilization, etc.). Depending on the QSAR selected, the toxicity calculation may be performed for fish, Daphnia or algae.

Aquatic Toxicity

(Gasoline): Ecotoxicity results for two blended gasolines (Concawe samples W94/813 and W94/814) have been evaluated and robust summaries for these samples tested as a water-accommodated fraction (WAF) have been prepared.

Concawe sample W94/813, (PONA 48-1-5-46) WAF induced a 96 hr LL_{50} = 11 mg/L (95% C.I. 9-16 mg/L) nominal loading rate in rainbow trout and a 48 hr. EL_{50} = 7.6 mg/L (95% C.I. 6.4-9.3mg/L) nominal loading rate in *Daphnia magna*. The algal 72 hr EL_{50} = 1.4 mg/L (95% C.I. 0-20 mg/L) in *Selenastrum capricornutum*.
Concawe sample W94/814, (PONA 40-12-6-41) WAF induced a 96 hr LL_{50} = 16 mg/L (95% C.I. 10-25mg/L) nominal loading rate in rainbow trout and a 48 hr. EL_{50} = 12 mg/L (95% C.I. 7.3-22 mg/L) nominal loading rate in *Daphnia magna*. The algal 72 hr EL_{50} = 4.2 mg/L (95% C.I. 0-24 mg/L) in *Selenastrum capricornutum*.

Biodegradation

(Gasoline):

Biodegradability of a commerical gasoline in aqueous medium was evaluated by measuring the disappearance of hydrocarbon constituents by gas chromatography with flame ionization detector, O_2 consumption (respirometry), and CO_2 production by gas chromatography with thermal

conductivity detector (Solena-Serena et al., 1999). Activated sludge microorganisms were found to biodegrade unleaded commercial gasoline up to 94% within 25 days. The carbon balance of gasoline degradation showed that 61.7% of gasoline was mineralized to CO2 and that microbial cell production accounted for the remaining carbon of gasoline degraded. For each hydrocarbon class, degradation occurred at different rates. Aromatic compounds were found to be the most readily consumed, although compounds bearing neighboring substituents and those containing longer alkyl groups were consumed at a slower rate than those with no or only one alkyl chain. Likewise, linear alkanes (exception for undecane), alkenes with five to nine carbons, cyclohexane and substituted cyclopentanes were biodegraded. Residual components of gasoline most recalcitrant to biodegradation were found to be branched alkanes, particularly those containing a quaternary carbon and/or alkyl chains on consecutive carbon atoms. The results of this study indicated that under the conditions of this test, the majority of gasoline constituents are rapidly and ultimately biodegraded by aquatic microorganisms.

Solano-Serena et al (1998) also evaluated the biodegradability of a representative gasoline prepared as a composite of 23 typical gasoline hydrocarbons by soil microflora suspended in aqueous media. The method of analysis of parent mixture, individual components, and CO_2 production was made by gas chromatography with flame ionization detector. The gasoline model mixture GM3 was degraded about 89% by a native soil suspension, based on GC/FID analysis of the initial and residual individual hydrocarbon concentrations. The results of this study indicated that the pattern of gasoline degradation was represented as the sum of the degradation of the individual compounds. No marked occurrence of co-metabolism was observed. Inhibitory effects were observed for 1,3,5 trimethylbenzene, 2-ethyltoluene and 1,2,3 trimethylbenzene at 200 mg/L, but were totally degraded at 35 mg/L by non-acclimated soil suspensions. The use of optimized degradative inoculum (soil microbes pre-exposed to cyclohexane and 2,2,4 TMP) in conjunction with non-acclimated soil organisms enhanced both rate and extent of the more structurally complex hydrocarbons that showed little to minimal degradation in non-acclimated soil systems.

APPENDIX 4

EU Categorization of Gasoline Blending Streams

This categorization of petroleum substances was adopted by the European Union in their legislation (Official Journal of the European Communities, L84 Volume 36, 5 April 1993. Council Regulation (EEC) N0 793/93 of 23 March 1993 on the evaluation and control of risks of existing substances). The organization of naphthas by PONA characteristics correlates well with the EU categorization which is based on the definitive process step to produce the stream, not on the final process step. The representative PONA-selected samples are listed in bold-face in the appropriate EU category. Although no samples were selected from Thermal Cracking (3E) and Hydrotreating (3F), these groups are adequately represented by the selected naphthas. Compositionally streams resulting from cracking under high temperature (3E) are similar to those derived from cracking using a catalyst (3D), and hydrotreating (3F) is employed with many streams to remove sulfur compounds and improve the quality of feedstock.

Gasoline Components from Crude Oil Distillation (3A)
Streams obtained from the atmospheric distillation of crude oil and containing saturated and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.
High Naphthenic: To be selected

Gasoline Components from Alkylation, Isomerisation and Solvent Extraction (3B)
Streams obtained by alkylation (catalytic reaction), isomerization (catalytic conversion) and solvent extraction, and containing saturated hydrocarbons, mainly in the range C5 to C12 and boiling in the range ca. 35 to 230°C.
High Paraffinic: Light Alkylate Naphtha, CAS #64741-66-8

Gasoline Components from Catalytic Cracking (3C)
Streams obtained from the catalytic cracking of heavy distillates into lighter fractions, and containing saturated, olefins and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.
High Olefinic: Light Catalytic Cracked Naphtha, CAS # 64741-55-5

Gasoline Components from Catalytic Reforming (3D)
Streams obtained from the catalytic reforming of mainly n-alkane and cycloparaffinic feedstocks into aromatic and branched chain hydrocarbons, mainly in the range C5 to C12 and boiling in the range ca. 35 to 230°C.
High Aromatic: Catalytic Reformed Naphtha, CAS # 68955-35-1

Gasoline Components from Thermal Cracking (3E)
Streams obtained by the high temperature splitting of heavy distillates into lighter fractions, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.

Gasoline Components from Hydrotreating (3F)
Streams obtained by the catalytic reaction of feedstocks with hydrogen to remove unsaturated and organo-sulphur compounds, and containing mainly saturated hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.

Other Gasoline Components (3G)
Streams obtained by processes such as steam and hydrocracking and sweetening, and containing saturated, aromatic and olefinic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca -20 to 230°C.

APPENDIX 5

Robust Summaries: Separate Documents

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # PARAFFINIC NAPHTHAS

Summary prepared by: American Petroleum Institute

Creation date: **30 NOVEMBER 2000**

Printing date: **24 OCTOBER 2001**

Date of last Update: **10 DECEMBER 2001**

Number of Pages: **32**

NB. Reliabiliy of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : petroleum product
Physical status : liquid
Remark : Paraffinic naphtha streams are obtained by alkylation (catalytic reaction), isomerization (catalytic conversion) and solvent extraction, and contain saturated hydrocarbons, mainly in the range C_5 to C_{12} and boiling in the range of approximately 35 to 230°C.

Light Alkylate Naphtha (CAS 64741-66-8) is a typical paraffinic naphtha stream.

Several samples have been used in testing for the toxicological properties of this group of substances. Details of three of the materials are given in the following table.

Sample (API 83-19) is a Light Alkylate Naphtha.
LAN is a Light Alkylate Naphtha.
LAND is a distillate of LAN.

	SAMPLE		
Parameter	API 83-19	LAN	LAND
Gravity (°API)	71.7		
Sulfur (wt %)	0.001		
RVP (psia)	6.7		
IBP (°F)	103		
FBP (°F)	331		
Olefins (% by MS)	0	0	0.03
Naphthenes (% by MS)	0.4	0.03	0
Aromatics (% by MS)	0.1	0	0
Saturates (% by MS)	99.5		
Paraffins		99.75	99.97
Mon (clear)	93.5		
RON (clear)	94.1		
Carbon No. (vol%)			
4		0.65	3.25
5		8.09	33.3
6		7.66	18.91
7		8.38	9.81
8		56.76	31.14
9		12.44	3.21
10		5.76	0.39

24.10.2001

2.2 BOILING POINT

Remark The paraffinic naphthas boil in the range of approximately 35 to 230 °C
One of the samples tested (API 83-19) had an initial boiling point of 107 °F and a final boiling point of 331 °F (equivalent to 39 and 166 °C respectively)

2.3 DENSITY

Type : relative density
Value : 0 .688 -0 .701 at 15° C
Method : ASTM D1298
GLP : no data
24.10.2001 (6) (7)

2.4 VAPOUR PRESSURE

Value : 0 430 - 4750 hPa at 37.8° C
Method ASTM D323
GLP : no data
24.10.2001 (6) (7)

2.5 PARTITION COEFFICIENT

Log pow : 3.11 - 4.54 at 25° C
Method calculated by LOGKOWWIN ver. 1.65
Year : 2000
GLP : no
Test substance : Light Alkylate Naphtha
Remark : Log P values represent the spread of calculated and/or measured values for the C_5 to C_9 hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.
Reliability : (2) valid with restrictions
26.06.2001 (15)

2.6.1 WATER SOLUBILITY

Method : Preparation of Water Soluble Fraction
Year : 1995
GLP : yes
Test substance : Light Alkylate Naphtha
Method : Water Accommodated Fractions (WAFs) of LAN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 72 hours in tightly closed systems with minimal headspace.
Result : Gas chromatographic analysis of selected components indicated freshwater and saltwater solubilities of 1.6 and 0.9 ppm respectively. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of 2,3 dimethyl butane; 2,4 dimethyl pentane;

2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Concentrations for these components reached equilibrium in freshwater and saltwater by 24 and 12 hours respectively.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LAN components range from <1 to approximately 30 mg/L. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.

Reliability : (2) valid with restrictions
26.06.2001 (9) (10) (11) (12) (19)

3.1.1 PHOTODEGRADATION

Type	:	calculation
Light source	:	Sun light
Method	:	calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	no
Test substance	:	Light Alkylate Naphtha
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Based on a 12-hour day, the range for atmospheric half-lives for LAN constituents is: 1.074 days (2,3,5 trimethyl hexane) to 15.985 days (isopentane).
Result	:	Indirect Photolysis: Yes Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 radicals/cm^3 Rate Constant: 0.6691E-12 (isopentane) cm^3/mol-sec to 9.956E-12 (2,3,5 trimethyl hexane) Half-life: 1.074 days to 15.985 days
Reliability	:	(2) valid with restrictions

26.06.2001 (16)

3.1.2 STABILITY IN WATER

Test substance	:	Light Alkylate Naphtha
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) valid without restriction

26.06.2001 (13)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	Calculated according to Mackay Level 1
Media	:	Soil, air, water, suspended sediment and sediment
Remark	:	Model based on chemical fugacity. Multimedia distribution was calculated for the C_5 to C_9 hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Mobility in the aquatic and terrestrial environment is low due to low water solubility and high vapor pressure. The naphtha components will partition rapidly to air, where for the majority of these hydrocarbons will be rapidly oxidized by OH radicals.
Result	:	

Medium	% distribution
Air	99.4 to 100
Soil	0.01 to 0.27
Water	0.001 to 0.02
Sediment	<0.001
Suspended sediment	<0.0

Conclusion	:	This complex petroleum mixture is expected to partition primarily to air
Reliability	:	(2) valid with restrictions

26.06.2001 (14)

3.5 BIODEGRADATION

Type : aerobic
Inoculum : mixed, adapted inoculum of domestic activated sludge and soil
Contact time : 56 day
Method : CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year : 1999
GLP : yes
Test substance : Light Alkylate Naphtha
Method : Test type:
Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO_2 production. By day 21 approximately 40% of the test material was degraded, a slight increase to 48% was observed by day 42, but by day 56 degradation had leveled back down to 40%. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63% actual) was observed by day 14, and total blank CO_2 production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21 °C, which deviated from the protocol value of 22 ±2°C. This deviation was not expected to have affected the outcome of this study.

	% Degradation (sd)	
Test Day	Hexadecane	Test Material
3	13.93 (1.85)	0.12 (0.07)
7	34.40 (4.54)	7.84 (7.80)
14	63.17 (0.94)	26.59 (0.85)
21	77.26 (6.52)	40.24 (5.00)
28	90.35 (7.14)	42.41 (2.54)
35	85.13 (n=1)	41.53 (9.90)
42	85.21 (n=1)	48.12 (1.77)
49	96.93 (8.94)	46.55 (1.04)
56	94.69 (4.10)	40.44 (0.76)

Test condition : Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two week adaptation period. Triplicate test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of

soil was obtained at a depth between 5-10cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substance were added directly to test systems using a 10 microliter Hamilton gas tight syringe. The volume required to achieve the specified mg carbon/l concentrations were calculated based on %carbon and specific gravity of the respective substance. The test substance %carbon (0.8505) and specific gravity (0.6690 mg/µl) information was supplied by the Sponsor. Hexadecane %carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/µl) was obtained from Verschueren (1983). Addition of respective substance was performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 (±- 2°C) in the dark.

Biodegradation by CO_2 determination-test initiation and procedure
On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume(100 ml inoculum/L). Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/lL concentration.
Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO_2 analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22°C (±- 2°).

On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc H_3PO_4 was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, then analyzed for CO_2 using gas chromatography-thermal conductivity detection. Quantitation of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability : (1) valid without restriction
24.10.2001 (18)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static with daily renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Year	:	1994
GLP	:	yes
Test substance	:	Light Alkylate Naphtha
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002. LL_{50} and LC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84. All NOEC values calculated using Fisher's exact test.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was Mobil Technical Center well water. Nominal loading rates of 0, 1.1, 5.2, 9.7, 19 and 74 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes. Fish were hatched and raised in-house, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8 liter glass containers with teflon lined caps. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving adequate volume to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

		Water temperature was 21.2 °C (0.2 °C sd). Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 7.7 and 8.6, pH values between 7.844 and 8.23.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 15, 20 and 20, respectively in 0, 1.1, 5.2, 9.7, 19 and 74 mg/L treatments. All surviving organisms exhibited normal behavior. 96-hr LL_{50} = 8.2 mg/l, 5.2-9.7 mg/L w/ 95% C.I. (as nominal loading rate) 96-hr LC_{50} = 305 ppb, 164-384 ppb w/ 95% C.I. (measured concentrations) 96-hr NOEL = 5.2 mg/l (as nominal loading rate) 96-hr NOEC = 166 ppb (measured concentrations)
Reliability	:	(2) valid with restrictions Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

24.10.2001 (21)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Year	:	1994
GLP	:	yes
Test substance	:	Light Alkylate Naphtha
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
	:	Statistical Method: (FT - ME) EL_{50} and EC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was aged well water. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 1.2 liters of water for 24 hr in aluminum foil covered 1 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25%or less vortex, the contents of the aspirator bottles were allowed to settle for approximately 45 minutes, then drained from the port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes. Range finding toxicity studies were conducted at 1.2, 9.9

and 99 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 9, 18, 35, 70, & 140 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.

Test vessels were teflon cap-sealed 237 ml glass jars with10 daphnids per jar and were completely filled with test solution.

During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.5; pH ranged from 8.00 to 8.2; temperature was 19.1 to 21.0 °C; hardness (mg/l) ranged from 180 - 204; alkalinity (mg/l) was 140-156 and conductivity (umhos) values were 385 - 390.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1994. The primary culture was obtained from Aquatic Research organisms, Hampton, NH, which was derived from EPA laboratory culture, in Cincinnati, Ohio.

Result : Immobility (no. of organisms) at 48 hrs: 0, 0, 0, 12, 13 and 20 for 0, 9, 18, 35, 70 and 140 mg/l treatments. At the 35 and 70 mg/ nominal treatments, 8 and 7 organisms were observed to show lethargic movement, respectively.

48-hr EL_{50} = 32 mg/l (95% C.I. 18 to 140 mg/l) based upon nominal loading rate.
48 hr EC_{50} was 556 ug/l (95% C.I. 339 to 1140 µg/l) based on total measured alkyl concentrations.

48-hr NOEL = 18 mg/l based upon nominal loading rate.

48 hr NOEC was 339 ppb based on total measured alkyl concentrations.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations

24.10.2001 (22)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Exposure period	:	96 hour(s)
Unit	:	Mg/L
Analytical monitoring	:	yes
Method	:	other: EPA. 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-8, ES-5.
Year	:	1995
GLP	:	yes
Test substance	:	Light Alkylate Naphtha
Method	:	Statistical Method: EL_{50} and EC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84. All NOEL/NOEC values calculated using Fisher's exact test.
Test condition	:	Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.3 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, pH adjusted to 7.5 ±0.1 with 0.1NHCl and sterilized by 0.22 micron filtration) in 2.0 liter aspirator bottles, covered with aluminum foil. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (135 ml) with treatment solution and inoculated with algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media, and transferred every 5-9 days to fresh media. Original algal cultures obtained from American Type Culture Collection (ATCC Strain 22662), Rockville, MD, June 1994. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 18, 70, 146, 292 and 1157 mg/l The initial algal concentration was 1.0 x 103 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 400 + 50-ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination Samples at 0,24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes. Test temperature was 24 ± 2°C. The pH was 7.5 at test initiation, pH value at test termination not included in report.

Result : Percent inhibition on growth determined by cell density (cells/mL):
96 hour EL_{50}=45mg/l (18-70 mg/l CI @95%)
96 hour EC_{50}=741ug/l (353-1060 ug/l CI @95%)
96 hour NOEL=18 mg/l
96 hour NOEC=741ug/l

Subcultures placed in fresh media (no test substance) after acute testing for nine days indicated that growth inhibition was algistatic in all treatments. No excursions from the protocol were noted. However, range finding and two previous definitive tests were performed and considered inconclusive due to inconsistencies in control and treatment cell densities, which presumably were resolved by modification of the AAP media. Additionally, control growth showed a lag during the first 48 hours of the study.

Nominal (mg/l) Conc	(meas µg/l).	96hr cell density (cells/ml)	(% Inhibition)
Control		$5.7x10^4$	na
18	(0.112)	$5.53x10^4$	3.1
70	(0.305)	$1.27x10^4$	77.7
146	(0.498)	$3.46x10^3$	93.9
292	(0.610)	$1.36x10^3$	97.6
1157	(0.612)	$1.60x10^3$	97.2

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitiated to determine total measured concentrations.

24.10.2001 (20)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	Undiluted
Value	:	> 7000 mg/kg bw
Year	:	1986
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	Groups of five male and five female fasted rats were given API 83-19 at doses of 5 and 7 g/kg as a single oral dose. The animals were then allowed food and water ad libitum and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded.
Result	:	Clinical signs seen during the study included: hypoactivity, diarrhea, yellow-stained anal area, red discharge from nose, blood-like discharge on or around penile area, pale appearance and one female in the 5 g/kg group died within one hour of dosing. All except two animals had returned to normal by day 3 of the study. The oral LD_{50} was found to be greater than 7 g/kg
Reliability	:	(1) valid without restriction

24.10.2001 (4)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	Air
Exposure time	:	4 hour(s)
Value	:	> 5 mg/l
Year	:	1987
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-19 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.
Result	:	The mean analytical and nominal exposure concentrations were 5.04 ± 0.74 and 6.31 mg/l respectively. All animals survived the study but exhibited languid behavior and a hunched appearance during the exposure.

Female body weights were decreased at day 15 but this was attributed to pre-necropsy fasting.
At necropsy there were no remarkable findings and histopathology of the lungs was normal.

Reliability : (1) valid without restriction
24.10.2001 (5)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	Undiluted
Value	:	> 2000 mg/kg bw
Year	:	1986
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to a dose of 2 g/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later and the animals were observed for a total of 14 days post-dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Result	:	A pain response (vocalization) was elicited from all the animals following application of the test material. Apart from skin irritation there were no other clinical signs of toxicity. Skin irritation ranged from slight to severe for erythema and edema, slight to moderate for atonia and coriaceousness and from slight to moderate for desquamation and fissuring. Subcutaneous hemorrhage, blanching and eschar was also observed.
Reliability	:	(1) valid without restriction

24.10.2001 (4)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Not relevant

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
PDII	:	3.9
Result	:	moderately irritating
Method	:	Draize Test

Year : 1986
GLP : yes
Test substance : API 83-19 (Light Alkylate Naphtha)
Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing.
After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.
Result : The scores for erythema and edema were marginally greater for intact skin than abraded skin, but the difference was not biologically significant.
Scores for intact skin at each of the observation intervals were:

Time	Erythema	Edema	Irritation score*
24 hours	2.0	1.7	3.5
72 hours	2.5	2.2	4.2
96 hours	2.7	2.8	4.9
7 days	2.5	2.3	4.5
14 days	0.8	1.0	1.2

* Irritation score calculated as the sum of irritation scores for each test site divided by the number of animals at each observation period.
PII is the sum of the 24- and 72- hour total irritation scores divided by 2

Reliability : (1) valid without restriction
24.10.2001 (4)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : 0.1 ml
Exposure Time : See method
Comment : Eyes rinsed 20-30 seconds after exposure (3 rabbits), Eyes not rinsed in remaining animals
Number of animals : 9
Result : not irritating
Year : 1986
GLP : yes
Test substance : API 83-19 (Light Alkylate Naphtha)
Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control.
After 20 to 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed.
Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result	:	No pain response was elicited on instillation of test material. No corneal or iridial irritation was seen during the study
Reliability	:	(1) valid without restriction

24.10.2001 (4)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	guinea pig
Concentration	:	Induction: 50 % occlusive, epicutaneous Challenge: 25 % occlusive, epicutaneous
Number of animals	:	10
Vehicle	:	paraffin oil
Result	:	not sensitizing
Year	:	1986
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	0.4 ml of a 50% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application, a challenge dose (0.4 ml of a 25% mixture in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream. Positive control (2,4-dinitrochlorobenzene), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups.
Result	:	At challenge, a very slight erythema was exhibited by one animal. The other 9 animals had no response. In contrast, all 20 of the positive controls responded with reactions ranging from slight to severe irritation. Only one naive control exhibited a very slight erythema upon challenge.
Reliability	:	(1) valid without restriction

24.10.2001 (4)

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	13 weeks
Frequency of treatment	:	6 hours/day, 5 days/week
Doses	:	668, 2220, 6646 ppm
Control group	:	Yes
NOAEL	:	= 2220 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1998
GLP	:	Yes
Test substance	:	The test material (LAND) was a distillate of a Light Alkylate Naphtha.

The compositions of the LAN and the distillate derived from it are shown in section 1.1. above.

The composition and uniformity chamber gas chromatic results (%weight) were:

Component	Liquid	Vapor	
		At Start	At termination
n-butane	2.442	3.217	3.210
iso-pentane	29.854	33.517	34.343
2,3-dimethylbutane	12.437	11.963	12.977
2-methylpentane	4.064	4.775	4.096
2,4-dimethylpentane	5.923	5.663	5.663
2,3-dimethylpentane	2.904	2.794	2.680
2,2,4-trimethylpentane	18.35	16.897	16.885
2,3,4-trimethylpentane	4.343	3.772	3.578
2,3,3-trimethylpentane	5.258	4.614	4.505
2,2,5-trimethylhexane	3.096	2.641	2.499

Chamber concentrations were monitored throughout the study. Actual chamber concentrations were close to target concentrations.
Particle mass distribution measurements confirmed that no measurable test material was present as aerosol.

Method : Groups of 12 male and 12 female rats underwent whole body exposures to 668, 2220 and 6646 ppm LAND. Exposures were for 6 hours each day, 5 days per week for 13 weeks.
Extra groups of 12 rats of each sex were exposed to the high dose level and for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 13 weeks exposure.

Neurobehavioral evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14 and 18 recovery groups. Animals were not exposed to LAND during these tests.

Following 13 weeks of exposure, 12 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically.

At the end of the 4 week recovery period, 12 animals of each sex from the high and control groups were necropsied and selected tissues were examined microscopically.

During the study, clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and 18 weeks (recovery groups). Body weights and food consumption was measured throughout the study.
Blood samples were taken from 12 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements.

At termination (after 13 weeks exposure for the main study and after 18 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) was removed from the control and high dose animals, were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination.
Nervous system tissues were selected randomly from 6 rats/sex/group in the high dose and controls at the end of 13 weeks for microscopic examination. Specific brain regions examined were forebrain, cerebral cortex, hipocampus, basal ganglia, midbrain cerebellum and pons and medulla.

Neurobehavioral studies were undertaken as follows:

Motor activity
Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 14 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery
An assessment of the following was made:

- Home cage evaluations for Posture, vocalization, palpebral closure.
- Handling evaluations for reactivity to general stimuli, signs of autonomic function.
- open field behavior: arousal level, gait, urination and defecation

frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.
Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result : There were no mortalities during the study and there were no treatment related signs of toxicity. A possible treatment related sign was an increased incidence of red facial staining in rats of both sexes in the high dose group.
Mean body weights, body weight gains and food consumption was unaffected by treatment.

Hematological and clinical chemical measurements were unaffected except for a 5% decrease in hemoglobin, a 5% decrease in hematocrit and a 7% decrease in erythrocytes. The hemoglobin was still decreased (4%) after the 4 week recovery period. However, it was considered that these differences were toxicologically unimportant because they were small and within the historical range for the test laboratory.

Although there were some changes in AST and ALT in high dose females they were not considered to be toxicologically significant because several control animals also had elevated levels for these enzymes in the control groups and also relative to historical controls.

The organ weight changes were few. Absolute and relative kidney weights were increased in the males at all dose levels and they were also elevated in the high dose recovery animals. These increases correlated with the finding of hyaline droplets in the proximal convoluted tubules at microscopy.
Absolute and relative liver weights were observed in the high dose males and females at 13 weeks but the differences had disappeared after the recovery period. There were no pathological findings associated with this increase.
The magnitude of the organ weight increases is shown below.

	Dose level (ppm)			
	668	2220	6646	Recovery
Males				
Abs. Kidney wt.	13.2	19.8	27	23
Rel. Kidney wt.		18	30	11
Abs. Liver wt.			21	
Rel. Liver wt.			25	
Females				
Abs. Liver wt.			17	12
Rel. Liver wt.			12	

In the neurobehavioral studies no treatment-related effects were observed in the functional operational battery.
In the study of motor activity, there were some statistically significant differences, but overall they did not occur in a dose related manner and furthermore were smaller than some of the differences seen during the pre dosing period.

Conclusion : LAND was not a neurotoxicant in the neurobehavioral studies that were conducted.
LAND did induce a light hydrocarbon nephropathy in the male

rats at all exposure levels, but this is regarded as species and sex specific and not relevant for human health risk assessment.
Excluding the nephropathy, the NOEL for subchronic toxicity was 2220 ppm and for neurotoxicity was 6646 ppm.

Reliability : (1) valid without restriction
24.10.2001 (17)

Species	:	rabbit
Sex	:	male/female
Strain	:	New Zealand white
Route of admin.	:	dermal
Exposure period	:	28 Days
Frequency of treatment	:	Once per day, three times per week for 4 weeks
Post obs. period	:	
Doses	:	200, 1000 & 2000 mg/kg
Control group	:	yes
Year	:	1986
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	Prior to the study, 5-day range finding study was conducted. The method and results of he range-finding study are not included in this summary. Undiluted API 83-19 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours, which was then removed, and the skin was wiped with a dry gauze to remove any residual material. A group of five rabbits of each sex served as sham controls. The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly. At termination, blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination. A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination.
Result	:	No deaths occurred during the study. During the latter half of the study, all but one high dose female looked thin. This was considered to be a treatment-related effect. Apart from skin irritation, there were no other treatment-related clinical signs. Weight gains of treated animals over the duration of the study was similar to controls except for the females at 2000 mg/kg. which were significantly reduced. The mean weight for these rabbits was the same at the end of the study as it was on day 1. A mean irritation score was calculated for each day and overall means were also calculated. (The mean irritation score, MIS, was the sum of irritation scores for both erythema and edema for all animals of a given dose group and sex.) The overall MIS for each dose group was:

Group/sex	MIS	Classification
2000 mg/kg M	3.5	Moderate irritant
2000 mg/kg F	3.6	Moderate irritant
1000 mg/kg M	2.8	Moderate irritant
1000 mg/kg F	2.7	Moderate irritant
200 mg/kg M	0.5	Minimal irritant
200 mg/kg F	0.5	Minimal irritant
Control M	0	Non irritant
Control F	0	Non irritant

There were no remarkable findings in the hematological data from any of the male or female groups compared to controls.
The only significant clinical chemical finding was an approximately 40% reduction in ALP of the 2000 mg/kg females. All other clinical chemical measurements were unremarkable.
There were few differences in organ weight between the control and treated animals, these were:

18% increase in R adrenal weight in 1000 mg/kg males
28% increase in L adrenal weight in 1000 mg/kg males
37% decrease in R ovary weight of 2000 mg/kg females.

In none of the above was there an associated change in the relative organ weights and the differences were not considered to be treatment-related.
At gross necropsy, treatment - related skin findings consisted of: dry, scaly, rough, fissured, reddened, crusted, and/or thickened skin. There were no other treatment-related findings at necropsy.
Although there were some findings at histopathology, they were not treatment-related except those in the skin.
The skin changes consisted of a slight to moderate proliferative and minimal to moderately severe inflammatory changes in the skin of all animals in the 2000 mg/kg groups. These skin changes were accompanied by an increased granulopoeisis of the bone marrow. This was considered to be possibly related to stress or other factors resulting from skin irritation.

Reliability : (1) valid without restriction
24.10.2001 (3)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Metabolic activation : with and without
Result : Negative
Year : 1985
GLP : Yes
Test substance : API 83-19 (Light Alkylate Naphha)
Method : The test material was dissolved in acetone for this assay.
Two positive control substances were used viz Ethyl methane sulphonate (EMS) at concentrations of 1.0 & 0.5 µl/ml and 7, 12-DMBA at concentrations of 7.5 & 5.0 µg/ml.

A cytotoxicity study was carried out prior to the mutagenicity assay. The results were difficult to interpret and as a consequence a second study was carried out and the results from this were used to determine the concentrations to be used in the subsequent lymphoma assay. It was established that complete toxicity occurred at 0.05 µl/ml for the non-activated cultures and at 0.5 µl/ml for S-9 activated cultures.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; TFT was used as the restrictive agent.

Eight non-activated and nine activated cultures were selected for cloning based on their degre of toxicity.
The non-activated cultures that were cloned were treated with 0.005, 0.01, 0.015, 0..02, 0.025, 0.03, 0.035 or 0.04 µl/ml and resulted in a range of growth of 6 to 97%. The activated cultures that were cloned were treated with 0.0002, 0.0009, 0.0028, 0.008, 0.02, 0.045, 0.09, 0.7 or 0.75 µl/ml and produced a range of growth from 24 to 109%.
Plates were prepared from TFT and from the VC cultures and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was then determined.

The following criteria were used in judging the significance of the activity of the test article.

Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Remark : Six mouse lymphoma assays were conducted but for technical reasons four of the assays were invalid.
In the fifth assay none of the cultures that were cloned, whether in the presence or absence of S-9 activation exhibited mutant frequencies that were greater than those for the solvent control. However, the toxic response in the S-9 activation portion of the assay was erratic and this portion of the assay was repeated.
This summary includes information from the fifth and sixth assays only, since they are the only ones considered to be valid.

Result : The results of the fifth assay are as follows:
After the 2 day recovery period, eight non-activated cultures and nine S-9 activated cultures were cloned based on their degree of toxicity.
The mutant frequencies and the percentage total growth at each of the test concentrations is summarised in the following table.

Concentration (µl/ml)	Mutant frequency	% Total growth
Non-Activated		
0.04	0	34
0.035	0.5	3
0.03	0.2	30
0.025	0	46
0.02	0	93
0.015	0.2	102
0.01	0	79
0.005	0	93
Solvent 1	0.5	
Solvent 2	0.6	
DMBA 7.5 µl/ml	3.6	27
DMBA 5 µl/ml	1.9	57

S-9 Activated		
0.75	0.2	101
0.7	0.2	16
0.09	0	88
0.045	-0.1	107
0.02	0	107
0.008	0.1	104
0.0028	0	100
0.0009	0	113
0.0002	-0.1	111
Solvent 1	0.6	
Solvent 2	0.6	
EMS 1µl/ml	8.7	3
EMS 0.5 µl/ml	6.8	29

The sixth assay was with S-9 activation only and the results were as follows:

S-9 Activated		
0.8	0.2	50
0.75	0	84
0.7	-0.1	90
0.65	-0.4	143
0.6	-0.1	99
0.5	-0.1	18
0.45	0.1	89
0.4	-0.1	72
0.35	0.1	76
0.25	-0.3	31
Solvent 1	0.8	
Solvent 2	0.8	
DMBA 7.5 µl/ml	1.4	62
DMBA 5 µl/ml	1.1	86

The authors concluded that according to the criteria used to judge the activity of the test material, the sample produced a negative response in the presence and absence of S-9 activation.

Reliability : (2) valid with restrictions
24.10.2001 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	i.p.
Exposure period	:	Up to 48 hours after single dose
Doses	:	0.3, 1.0 & 3.0 g/kg
Result	:	negative
Year	:	1985
GLP	:	yes
Test substance	:	API 83-19 (Light Alkylate Naphtha)
Method	:	The study design was as follows:

Treatment	Animals/sex/scrifice time		
	6 hrs.	24 hrs.	48 hrs
Corn oil (vehicle)	5	5	5
API 83-19, 3 g/kg	5	5	5
API 83-19, 1 g/kg	5	5	5
API 83-19, 0.3 g/kg	5	5	5
Triethylenemelamine (Positive control)		5	

Test material in vehicle was given intraperitoneally at a dose of 5 ml/kg to groups of rats as shown above. Corn oil was used as vehicle control and TEM (0.5 mg/kg) as the positive control
Two to four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1 mg/kg).
2 Males and one female in the high dose group died, these were replaced by substitute animals that were killed approximately 50 hours after administration of the test material.
Immediately after sacrifice bone marrow was obtained from the femurs of the animals. The marrow was washed and the cells were fixed before being spread on slides (at least 3 from each animal) for examination.
Slides were scored for chromosomal aberrations.
Where possible, a minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy (1-3).
A mitotic index (= No. of cells in mitosis/500 counted X 100) was calculated and recorded.

The data were evaluated according to the following criteria:
For the test to be considered to be valid, the % of cells in the negative control group demonstrating aberrations of any type, other than gaps, must not exceed 4%.
The % of cells with aberrations in the positive control group must be statistically increased ($p \leq 0.05$) relative to the vehicle control using Chi-square statistics.

The test material is considered positive when the % of cells with aberrations in any treatment group is significantly increased ($p \leq 0.05$) relative to the vehicle control using Chi-square analysis and the number of aberrations per

cell is also significantly increased (p < or = 0.05) relative to the vehicle control using t-test statistics.

Result : The dose levels used in the assay were selected on the basis of a preliminary screen in which only one male rat died within 24 hours following the administration of API 83-19 as a single i.p. dose to 4 rats of each sex.
In the cytogenetics assay, 5 of 18 males and 4 of 18 females receiving 3 g/kg API 83-19 died within 3 days. At this dose level, there was a weight loss of 10% and 9% in males and females respectively within 48 hours of administration.
Other signs of toxicity included piloerection, crusty eyes and noses and excess lacrimation.
No sex-related differences were noted in the study and therefore the data for males and females were combined for the cytogenetics evaluation. The results are summarized in the following table.

	0.3 g/kg	1 g/kg	3 g/kg	Positive	Vehicle
Cells with aberrations					
6 hrs	0	2	0		0
24 hrs	1	0	1	171	0
48 hrs	1	0	1		0
Incidence of aberrations (%)					
6 hrs	0	0.4	0		0
24 hrs	0.2	0	0.2	34.2	0
48 hrs	0.2	0	0.3		0
No. Gaps					
6 hrs	0	2	0		0
24 hrs	0	0	0	15	1
48 hrs	0	0	4		1
No. Breaks					
6 hrs	0	2	0		0
24 hrs	1	0	1	197	0
48 hrs	2	0	1		0
Aberrations per cell					
6 hrs	0	0.004	0		0
24 hrs	0.002	0	0.002	2.336	0
48 hrs	0.004	0	0.003		0

NB.1. 500 cells were evaluated for each time point at each dose level.
NB.2. In the API 83-19 and vehicle control groups no rearrangements were observed and no aberrations from severely damaged cells were seen. In contrast, 51 rearrangements and 920 aberrations from severely damaged cells were seen in the positive control group.

Reliability : (1) valid without restriction
24.10.2001 (1)

5.8 TOXICITY TO REPRODUCTION

Type	:	One generation study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hours per day
Frequency of treatment	:	Daily
Premating exposure period		
Male	:	14 days
Female	:	14 days
Duration of test	:	females 7 weeks, males 8 weeks
Doses	:	5, 12.5 and 25 g/m³
Control group	:	yes
NOAEL Parental	:	> 24700 mg/m³
NOAEL F1 Offspr.	:	> 24700 mg/m³
Method	:	Adaptation of OECD No. 421
Year	:	1998
GLP	:	yes
Test substance	:	Distillate of light alkylate naphtha

The test material was prepared to be representative of the fraction of light alkylate naphtha to which man would normally be exposed during normal handling and use.

The test material (LAND) was obtained by the distillation of light alkylate naphtha (LAN) and collecting that fraction that boiled over the temperature range 78 to 145°F.
The sample was analyzed and its composition compared to the light alkylate naphtha from which it was derived.
The compositions of the distillate and starting material were as follows:

Compound	Weight %	
	LAND	LAN
n-butane	3.42	0.84
isopentane	63.59	12.61
n-pentane	1.33	0.23
2,3-dimethylbutane	22.51	4.74
2-methylpentane	6.44	1.57
3-methylpentane	2.26	0.74
2,4-dimethylpentane	0.29	4.09
2,2,4-trimethylpentane	0.06	23.92
2,3,3-trimethylpentane	0	8.99
2,3,4-trimethylpentane	0	11.56

Method : The test material was totally vaporized and diluted with air to achieve the desired concentrations for the study.
Exposures were conducted in one cubic meter whole-body chambers.
Chamber concentrations were monitored three times daily by GC/FID.
All animals were housed individually in suspended mesh cages.

10 animals of each sex were exposed 6 hours each day to test material at target concentrations of 5, 12.5 and 25 g/m³.

The animals were exposed for 6 hours each day.
Parental females were exposed for 14 days prior to mating, throughout mating and gestation days 0-19 (7 consecutive weeks). Dams and their litters were sacrificed on postpartum day 14. Parental males were also exposed for 14 days prior to mating, during mating, throughout the female gestation and post partum period and throughout the female necropsy period (8 consecutive weeks).
Rats were mated in a 1:1 ratio and females were monitored for evidence of mating by the examination of a vaginal lavage sample for sperm or vaginal plug. If sperm or a vaginal plug were observed, the female was considered to be at day 0 of gestation and the male was removed from the female at this stage. If there was no evidence that mating had occurred the pairs were allowed to remain together up to a period of 2 weeks after which time the female was assumed to be pregnant.
All animals were observed for clinical signs at least twice daily throughout the study. Body weights and food consumption were recorded throughout the study.
Each litter was examined as soon as possible after delivery to establish number and sex of pups, stillbirths, live births and presence of gross abnormalities. Neonatal survival was monitored and all pups were killed postpartum days 4 or 5.
Parental females were killed on gestation day 25 if they had not delivered, otherwise they were killed on postpartum days 4 or 5.
At necropsy each parental animal was examined macroscopically for structural abnormalities and pathological changes with emphasis on reproductive organs. Additionally the number of implantation sites and corporea lutea of each female were recorded. Lungs, trachea and larynx were removed in their entirety. The right middle lobe of the lung was weighed, the remaining lobes were fixed for subsequent histopathological examination. The testes and epididymes of the males were weighed and then fixed for histological examination as were the ovaries of the females.

Result **:** The chamber concentrations of test material were found to be between 96 and 104% of nominal, the mean highest dose concentration being 24.7 mg/m³.
The vapor compositions were also found to be similar to that of the parent test material.
No parent animals died or were killed during the study and there were no clinical signs. Body weights and food consumption were unaffected by exposure to test material.
Results on reproductive capacity and fertility are summarized in the following table.

	Treatment group 9g/m³			
Parameter	0	5	12.5	25
Pregnancy (%)	80	80	100	80
Litters with live pups	8	8	9	8
Implantation sites	14.9	16.8	13.9	17.3
Pups delivered	14.4	15.6	14.3	15.6
Live pups/litter	14.4	14.8	13.8	15.5
No. liveborn	115	118	124	124
Live birth index (%)	100	94	96	99
Pups surviving 4 days	113	114	122	123
Viability index (%)	98	97	98	99
Litter wt day 1	7.2	7.3	7.1	7.1
Litter wt day 4	10.8	11.1	11.2	10.5

There were no treatment-related findings observed at necropsy . Organ weights were unaffected by treatment and there were no treatment-related histological findings.

Reliability : (1) valid without restriction
24.10.2001 (8)

(1) American Petroleum Institute (1985)
Acute In Vivo cytogenetics assay in male and female rats of API sample 83-19.
Study conducted by Microbiological Associate Inc.
API Health & Environmental Sciences Department, Publication No. 32-32409

(2) American Petroleum Institute (1985)
L5178Y +/- Mouse lymphoma assay, API 83-19 Light Alkylate Naphtha. Study conducted by Microbiological Associates Inc.
API Health and Environmental Sciences Dept. Report 32-32746

(3) American Petroleum Institute (1986)
28-Day dermal toxicity study in the rabbit. API 83-19, Light alkylate naphtha (CAS 64741-66-8). Study conducted by Tegeris laboratories.
API Health and Environmental Sciences Dept. Publ. 33-30498

(4) American Petroleum Institute (1986)
Acute oral toxicity study in rats, acute dermal toxicity study in rabbits, primary dermal irritation study in rabbits, primary eye irritation study in rabbits, dermal sensitization study in guinea pigs on API 83-19, Light Alkylate Naphtha (CAS 64741-66-8). Study conducted by Hazleton Laboratories.
Health and Environmental Sciences Dept. Report 33-30594

(5) American Petroleum Institute (1987)
Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats API 83-19 Light Alkylate Naphtha (CAS 64741-66-8). Study conducted by Hazleton laboratories.
API Health and Environmental Sciences Dept. Report 34-30636

(6) American Petroleum Institute (1987)
Comprehensive analytical analysis of API generic refinery streams.

(7) American Society for Testing and Materials (ASTM), 1991
Annual Book of ASTM Standards. Section 5, Petroleum Products, Lubricants and Fossil Fuels, ASTM, Philadelphia, Pa., 1991.

(8) Bui, Q., Burnett, D. M., Breglia, R. J., Koschier, F. J., Lapadula, E. S., Podhasky, P. I., Schreiner, C. A. and White, R. D. (1998)
Toxicity evaluation of petroleum blending streams: reproductive and developmental effects of a distillate from light alkylate naphtha.
J. Tox. Env. Health, Part A, Vol 53, pp 121-133

(9) Concawe (1992)
Ecotoxicological Testing Of Petroleum Products : Test Methodology.
Report 92/56,
CONCAWE, Brussels.

(10) Concawe (1996)
Environmental risk assessment of petroleum substances: the hydrocarbon block method.
Report 96/52,
CONCAWE, Brussels.

(11) ECETOC (1998)
QSARS in the Assessment of the Environmental Fate and Effects of Chemicals. Technical Report No. 74

(12) ECETOC, (1996)
Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances. Monograph 26.

(13) Harris, J.C. (1982)
Rate of Hydrolysis. In Handbook of Chemical Property Estimation Methods. p. 7-6. W. J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY, USA.

(14) Mackay, D, A. DiGuardo, S. Paterson, & C. Cowan (1997)
EQC Model, ver. 1.01, 1997, available from the Environmental Modelling Centre, Trent University, Canada.

(15) Meylan, M, SRC 1994-1999.
LOGKOWWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(16) Meylan, M, SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(17) Schreiner, C., Lapadula, E., Breglia, R., Bui, Q., Burnett, D., Koschier, F., Podhasky, P. and White,R. (1998)
Toxicity evaluation of petroleum blending streams: inhalation subchronic toxicity/neurotoxicity study of a light alkylate naphtha distillate in rats.
J. Toxicol. and Env. Health, Part A, Vol 55, pp 277-296

(18) Springborn Laboratories, Inc. (1999)
Light Alkylate Naphtha-Determination of Inherent Biodegradability. Study No. 13687.6111

(19) Stonybrook Laboratories, Inc. (1995)
Method Validation for the Analysis of Whole Light Alkylate Naphtha (LAN) in Water Accomodated Fraction (WAF) using Purge-and-Trap and GC/FID, Study No. 65969.
Stonybrook Laboratories Inc. Princeton, NJ.

(20) Stonybrook Laboratories, Inc. (1995)
Static Renewal 96-hour acute toxicity of the water accomodated fraction (WAF) of Whole Light Alkylate Naphtha (LAN) Product to a Freshwater Alga, Selenastrum capricornutum, Study No. 65909.
Stonybrook Laboratories Inc. Princeton, NJ.1995.

(21) Stonybrook Laboratories, Inc. (1995)
Static Renewal 96-hour acute toxicity of the water accomodated fraction (WAF) of Whole Light Alkylate Naphtha (LAN) Product to Fathead Minnow. Study No. 65908.
Stonybrook Laboratories, Inc. Princeton, NJ.1995.

(22) Stonybrook Laboratories, Inc. (1995)
Static Renewal 48-hour acute toxicity of the water accomodated fraction (WAF) of Whole Light Alkylate Naphtha (LAN) Product to Daphnia Magna. Study No. 65907.
Stonybrook Laboratories Inc. Princeton, NJ.

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # GASOLINE

Summary prepared by: American Petroleum Institute

Creation date: **13 MARCH 2001**

Printing date: **11 DECEMBER 2001**

Date of last Update: **11 DECEMBER 2001**

Number of Pages: **49**

NB. Reliabiliy of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : petroleum product
Physical status : liquid
Remark : Gasoline is a complex combination of hydrocarbons consisting primarily of paraffins, cycloparaffins, aromatic and olefinic hydrocarbons having carbon numbers predominantly greater than C3 and boiling in the range of 30°C to 260°C.

To achieve acceptable physical and combustion properties, gasoline is prepared by blending naphtha streams, each of which will consist predominantly of one or other chemical type.
The streams normally used are:

Paraffinic streams derived from alkylation, isomerization and solvent extraction

Olefinic streams derived from catalytic cracking

Naphthenic streams derived from crude oil distillation

Aromatic streams derived from catalytic reforming

The American Petroleum Institute prepared a blend of naphtha streams which was considered to be typical of US gasoline in the middle 1970s. The blend was designated PS-6 gasoline. The benzene
content of PS-6 was adjusted upwards to 2.0% by volume, although this would never happen in normal refinery practice. The proportions of the various naphtha streams used to prepare the PS-6 gasoline blend were:

Naphtha stream/Component	% vol.
Paraffinic naphtha streams	22.0
Olefinic naphtha streams	52.1
Aromatic naphtha streams	21.3
Benzene	0.8
n-butane	3.8

A more comprehensive characterization of PS-6 gasoline was reported by MacFarland et al. as follows:

Research Octane No.	92.0
Motor Octane No.	84.1
Reid vapor pressure (Lbs)	9.5
Distillation	
Initial boiling point (°C)	93
Final boiling point (°C)	428
API Gravity	60.6
Gum, ASTM D381 (mg/gal	1
Sulfur (ppm)	97
Phosphorus (g/gal)	<0.005
Lead (g/gal)	<0.05
Stability (hrs)	>24
Hydrocarbon analysis, ASTM D1319	
Aromatics (vol%)	26.1
Olefins (vol%)	8.4

Saturate (vol%)	65.5
Benzene (%)	2.0

An unleaded gasoline, tested in an inhalation teratology study had the following physical chemical characteristics:

API Gravity	57.4
Reid vapor pressure (Lbs)	8.8
Initial boiling point (°C)	88
Final boiling point (°C)	378
Sulfur (ppm)	157
Vapor density	3.4
Paraffins (vol%)	47
Olefins (vol%)	4
Naphthenes (vol%)	10
Aromatics (vol%)	39
C6 Aromatics (%)	1.2
C7 Aromatics (%)	10.2
C8 Aromatics (%)	16.3
C9-plus Aromatics (%)	11.3

19.11.2001 (22)

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 300 ppm
Short term exposure
Limit value : 500 ppm
Schedule : 8 hour(s)

04.07.2001 (1)

2.1 MELTING POINT

Not relevant

2.2 BOILING POINT

See section 1.1 (General substance information)

2.3 DENSITY

Type	:	relative density
Value	:	ca. 50
Year	:	1984
Test substance	:	API PS-6 gasoline
Reliability	:	(1) valid without restriction

18.11.2001 (24)

2.5 PARTITION COEFFICIENT

Log pow	:	= 2.13 - 4.5 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65.
Year	:	2000
GLP	:	no
Test substance	:	Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Remark	:	Log P values represent the spread of calculated and/or measured values for C_5 to C_8 hydrocarbon components found in gasoline, CAS No 86290-81-5. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability	:	(2) valid with restrictions

19.11.2001 (13) (26)

Log pow	:	= 2.73 - 4.85 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65.
Year	:	2000
GLP	:	No
Test substance	:	Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.4 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 µg/l EDTA
Remark	:	Log P values represent the spread of calculated and/or measured values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability	:	(2) valid with restrictions

21.11.2001 (13) (26)

2.6.1 WATER SOLUBILITY

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	yes
Test substance	:	Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Method	:	Water Accommodated Fractions (WAFs) of LSRN were prepared at 100 mg/l loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of BTEX components indicated freshwater solubility at 24 hours of 3.1, 3.1, <6.9E-3, and 0.92 ppm (as BTEX, respectively).
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) valid with restrictions

19.11.2001 (7) (14) (15) (16) (30)

3.1.1 PHOTODEGRADATION

Type	:	Calculated
Light source	:	Sun light
Indirect photolysis		
Sensitizer	:	OH
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	% after
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	No
Test substance	:	Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_8 hydrocarbon components found in gasoline. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. Based on a 12-hour day, the range for atmospheric half-lives for gasoline constituents is: 0.789 days (m-xylene) to 15.985 days (isopentane).
Result	:	Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 OH radicals/cm3 Rate Constant: 0.6991 E-12 (isopentane) to 13.5606 E-12 (m-xylene) cm^3/molecule-sec Half-life: 0.789 to 15.985 days
Reliability	:	(2) valid with restrictions

19.11.2001 (13) (27)

3.1.2 STABILITY IN WATER

Test substance	:	Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) valid without restriction

19.11.2001 (17)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	Calculated
Media	:	Soil, air, water, suspended sediment, sediment
Method	:	Calculated according to Mackay Level I
Year	:	2000
Remark	:	Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_8 hydrocarbon components found in gasoline. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. The majority of components in gasoline will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals. With the exception of toluene, partitioning to air is > 97% for all components.

Result : Medium / % distribution
Air: 97 to 99.99
Soil: 0.00 to 1.2
Water: 0.003 to 2.7
Sediment <0.001 to 0.02
Suspended sediment <0.001 to 0.02

Test substance : Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6)

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air. Moderate partitioning to water and soil is predicted for the aromatic components of this mixture

Reliability : (2) valid with restrictions
19.11.2001 (13) (23)

3.5 BIODEGRADATION

Type : Aerobic

Inoculum : Activated aerobic sludge obtained from an urban wastewater treatment plant.

Deg. Product :

Method : Non-guideline research method using a closed-system shake flask apparatus.

Year : 1999

GLP : No data

Test substance : CAS No. 86290-81-5; a commercial unleaded gasoline topped at 76°C by distillation

Method : Aerobic Biodegradabilty - Evaluation of biodegradability of gasoline in aqueous medium. Method by analysis of disappearance of carbon compounds (gas chromatography with flame ionization detector), kinetics of O_2 consumption (respirometry), and CO_2 production (gas chromatography with thermal conductivity detector).

Exposure period was 16 or 25 days.
See test conditions for more details.

Result : Biodegradation and Mineralization of Gasoline
Gasoline was degraded up to 94% under non-limiting conditions after 25 d incubation (500 ml substrate/l medium). The carbon balance of gasoline degradation showed that 61.7% of gasoline was mineralized to CO_2 and that microbial cell production accounted for the remaining carbon of gasoline degraded. Biomass formation and mineralization occurred mainly during the initial fast degradation phase whereas essentially mineralization occurred during the second slow degradation phase. Individual classes of hydrocarbons degraded and carbon balance were shown to be:

Hydrocarbon Class	Amount in Gasoline mg/g	After 2 days	After 25 days
Aromatics	789	88%	99%
Branched alkanes	165	14	74
Linear alkanes	23	17	92
Cyclic alkanes	17	10	99
Alkenes	6	71	99

Carbon balance

Substrate or Products	Initial amount (mg C/l)	Final amount (mg C/l)
Gasoline	357	18
Biomass	39	165
CO2	0	204
Total Carbon	396	387

Kinetic Experiments with Gasoline
Two main degradation phases were found, one fast degradation phase (FDP), which started after an 18 h lag period and lasted until the 40th hour. The maximum rate of oxygen consumption during the FDP was 44 mg/l/h and the average rate was 24.5 mg/l/h. The FDP was followed by a slow degradation phase (SDP) where the rate of oxygen consumption slowed steadily from the 40th hour until the 25th day. The average rate was 15 mg/l/d, which was approximately 40 times slower than during the FDP.

Test condition : Activated sludge containing approximately 3 g/l dry weight was centrifuged at 15000 g for 20 min and re-suspending the biomass in the same volume of nutrient solution. The microbial suspension was used to inoculate nutrient solution at a final concentration of 100 mg dry weight/l. Gasoline (400 mg/l) or individual hydrocarbons (150 mg/l) were added to the medium as the sole carbon source. The nutrient solution was a vitamin-enriched mineral salt medium described by Bouchez et al. Appl. Microbiol. Biotechnol. 43:156-164 (1995).

Biodegradation of Gasoline
The biodegradation tests were performed in 500-ml flasks with sidearms equipped with Mininert© valves. 25 ml of gasoline were added to 50 ml of inoculated nutrient medium (i.e., 500 ml substrate/l medium) through the valve with a syringe. The flasks were incubated for 25 days at 30°C with alternate shaking (70 strokes per min). After the incubation period, 5 ml of CH_2Cl_2 containing 600 mg/ml dodecane as internal standard was introduced to the flasks through the valve, and the remaining hydrocarbon compounds were extracted for 1 h under shaking. The flasks were refrigerated overnight at 4°C before opening. The suspensions were centrifuged at 35000 g for 30 min at 4°C. The CH_2Cl_2 phase of each flask was then analyzed by gas chromatography for carbon compounds. Experiments were performed in duplicate and abiotic controls were prepared similarly to the other treatments with the exception that 1 g/l $HgCl_2$ were added to the flasks before incubation.

Mineralization of Gasoline
Measurements of CO_2 evolved during the biodegradation of gasoline were conducted in 240-ml flasks closed by Viton © stoppers. 18 ml of inoculated culture medium were added to each flask along with 5 ml of gasoline (i.e., 500 ml substrate/l medium). Flasks were incubated at 30°C for 25 days under alternate shaking. At the end of the incubation period the contents of each flask was acidified with 0.5 ml HNO_3 (68%) and CO_2 was measured by gas chromatography. Endogenous respiration of inoculated medium was measured in flasks without gasoline added.

Kinetic Experiments with Gasoline
Kinetics of O_2 consumption during gasoline biodegradation were determined in duplicate at 30°C over 25 d by respirometry. 500 ml stirred culture flasks contained 250 ml of inoculated nutrient medium and 125 ml of gasoline (i.e., 500 ml substrate/l medium). Control experiments without gasoline were also done. Kinetics of hydrocarbon degradation also was monitored by respirometry. Incubation was stopped at selected times and the remaining hydrocarbons were extracted as described above and analyzed by gas chromatography.

Kinetic Experiments with Individual Hydrocarbons
Kinetics of CO_2 production during the degradation of individual hydrocarbons was carried out at 30°C over 16 days. Treatments were prepared in 125 ml shaken flasks with 25 ml of nutrient solution containing 70 mg/l of inoculum biomass and 5 ml of hydrocarbon (i.e., 200 ml substrate/l medium). Flasks were closed with Teflon-coated stoppers and sealed. CO_2 was measured at various times by gas chromatography. Endogenous respiration was determined in flasks without hydrocarbon added.

Test substance : CAS No. 86290-81-5; a commercial unleaded gasoline topped at 76°C by distillation. It was free of hydrocarbons having less than six carbon atoms and contained no oxygenated compounds.

Conclusion : Activated sludge microorganisms were found to biodegrade unleaded commercial gasoline up to 94% within 25 days. For each hydrocarbon class, degradation occurred at different rates. Aromatic compounds were found to be the most readily consumed, although compounds bearing neighboring substituents and those containing longer alkyl groups were consumed at a slower rate than those with no or only one alkyl chain. Likewise, linear alkanes (exception for undecane), alkenes with five to nine carbons, cyclohexane and substituted cyclopentanes were biodegraded. Residual components of gasoline most recalcitrant to biodegradation were found to be branched alkanes, particularly those containing a quaternary carbon and/or alkyl chains on consecutive carbon atoms.

Reliability : (2) valid with restrictions
21.11.2001 (29)

Type :
Inoculum : Native soil suspension (NSS) from a spruce forest and microbially-reinforced native soil suspension (MRSS).
Method : Non-guideline research method using a closed-system shake flask apparatus
Year : 1998
Test substance : CAS No. 86290-81-5; artificially-prepared produced gasoline-model mixture (GM23)
Method : Soil biodegradation - Evaluation of biodegradability of GM23 by soil microflora; mineralization of GM23; and mineralization of 23 principal components of GM23. Method of analysis of parent mixture, individual components, and CO_2 production made by gas chromatography with flame ionization detector.

Exposure period: 14, 28 & 34 days

See test condition for full description.

Result : The gasoline model mixture GM23 was degraded about 89% by a

native soil suspension, based on GC/FID analysis of the initial and residual individual hydrocarbon concentrations. Cyclohexane, 2,2,4-trimethylpentane and 2,3,4-trimethylpentane were only slightly biodegraded and 3-methylhexane, 2,4-dimethylhexane, and 1,3,5-trimethylbenzene were incompletely biodegraded. All other compounds were consumed by NSS. Supplementation of NSS with cyclohexane-degrading and 2,2,4-trimethylpentane-degrading microflora led to complete degradation of GM23.

Biodegradation and mineralization of GM23 at 28 days
Mineralization yields were:
MRSS inoculum: 0.58 (58%)
NSS inoculum: 0.45 (45%) .

Kinetic Experiments with GM23
Consumption of each component by NSS did not occur at the same rate. n?alkanes and most of the mono-aromatic compounds were completely biodegraded within the first 14 days, whereas cyclohexane and di- and trimethyl alkanes were not. Methyl alkanes and dimethyl hexanes were slightly consumed over this period of time, but they were nearly totally biodegraded after 28 days. Cyclohexane and trimethyl pentanes showed little biodegradation after 28 days.

Mineralization Experiments with Individual Hydrocarbons
The mineralization yields of individual components of GM23 were high for the aromatics and alkanes with no or few methyl groups. Furthermore, the mineralization yield of GM23 determined with NSS or MRSS agreed with the mineralization data for individual components. The ability of the NSS to mineralize individual hydrocarbons indicated:

Substrate	Mineralization Yield at 34 days
heptane	0.74
octane	0.49
cyclohexane	0
2-methylhexane	0.47
3-methylhexane	0.71
3-methylheptane	0.69
2,4-dimethylhexane	0.56
2,5-dimethylhexane	0.44
2,2,4-trimethylpentane	0.02
2,3,4-trimethylpentane	0
benzene 0.56toluene	0.63
o-xylene	0.49
m-xylene	0.61
p-xylene	0.48
ethylbenzene	0.48
n-propylbenzene	0.34
2-ethyltoluene	0
3-ethyltoluene	0.38
4-ethyltoluene	0.34
1,2,3-trimethylbenzene	0
1,2,4-trimethylbenzene	0.18
1,3,5-trimethylbenzene	0

Test condition : The soil sample originated from a spruce forest (Foulain, France) and contained mineral and superficial organic matter. The NSS soil suspension was prepared with 20 g of homogenized soil per liter of nutrient solution. The MRSS soil suspension contained a cyclohexane-degrading microflora

and a 2,2,4-trimethylpentane-degrading microflora obtained from gasoline-polluted sites and added at 5% v/v each to the NSS. The nutrient solution contained minerals and vitamins prepared as described in Bouchez et al. Appl. Microbiol. Biotechnol. 43:156-164 (1995).

Biodegradation Experiments with GM23
The biodegradation tests were performed with NSS and MRSS suspensions (50 ml) in 500-ml flasks with sidearms equipped with MininertÒ valves. 25 ml of GM23 were added to 50 ml of soil suspension through the MininertÒ valve with a syringe (500 ml substrate/l medium). After an incubation period of 14 or 28 days at 30°C, 5 ml of CH_2Cl_2, containing dodecane at 600 mg/ml as internal standard, were introduced in the flasks through the valve, and the remaining hydrocarbons were extracted for 1 h with shaking. The flasks were refrigerated overnight at 4°C before opening. The suspension was centrifuged at 4°C and 35000 g for 30 min. The CH_2Cl_2 phase of each flask was then analyzed by gas chromatography. Mineralization yields were estimated at the end of the biodegradation experiment by recovery of CO_2 after acidification of the flask contents. Abiotic treatments were prepared similarly to the other treatments with the exception that 1 g/l $HgCl_2$ were added to the flasks before incubation.

Kinetic Experiments with GM23
Kinetics of CO_2 production during the degradation of GM23 were studied at 30°C for 28 days. 18 ml of NSS or MRSS were introduced into 240-ml flasks that were sealed by stoppers covered with Teflon film. 8 ml of GM23 were dispensed into the sealed flasks by syringe (444 ml substrate/l medium). A 250-ml gas-tight syringe was used to sample head space gas. Endogenous respiration of NSS and MRSS were determined under the same conditions in flasks incubated without test substance.
Mineralization Experiments with Individual Hydrocarbons
Mineralization yields of the individual hydrocarbon compounds were determined using 125-ml flasks containing 20 ml of soil suspension and 5 ml of each hydrocarbon (250 ml substrate/l medium). Flasks were incubated for 34 days. Two flasks for each compound and six control flasks were used.

Test substance : CAS No. 86290-81-5; artificially-prepared produced gasoline-model mixture (GM23) of main compounds making up a topped gasoline-cut obtained by distillation at 76°C of a French commercial unleaded gasoline. The model mixed gasoline was free of compounds lighter than C_6 and contained no oxygenated compounds. Biodegradation also was measured on 23 individual components of the model gasoline.

Conclusion : The use of optimized degradative inoculum (soil microbes pre-exposed to cyclohexane and 2,2,4 TMP) in conjunction with non-acclimated soil organisms enhanced both rate and extent of the more structurally complex hydrocarbons which showed little to minimal degradation in non-acclimated soil systems.
The results of this study indicated that the pattern of gasoline degradation was represented as the sum of the degradation of the individual compounds. No marked occurrence of co-metabolism was observed. Inhibitory effects were observed for 1,3,5 trimethylbenzene, 2-ethyltoluene and 1,2,3 trimethylbenzene at 200 mg/l, but were totally

degraded at 35 mg/l by non-acclimated soil suspensions.

Reliability : (2) valid with restrictions acceptable, well-documented publication which meets basic scientific principles

16.11.2001 (28)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : LL_{50} at 96 hr calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84
Result : Mortality (no. of deaths/treatment) at 96 hrs:

Treatment (mg/l)	No. of deaths
0	0
0.1	0
1.0	0
5.0	0
10	0
25	15

96-hr LL_{50} = 16 mg/l, 99% C.I: 10-25 mg/l (as nominal loading rate)
96-hour No Observed Effect Loading (NOEL) was 10 mg/l, based on mortality, both calculated (Dunnett's Procedure) and observed.
Results are quoted in terms of 50% Lethal Loading (LL_{50}), the loading rate of test substance resulting in 50% mortality of the test species exposed to the WAF. At termination, loss of equilibrium was observed in all surviving fish at the 10 mg/l treatment.

Analytical results
Losses of the soluble BETXN components from the WAF over each 24 hour period ranged from 0 to 8% for the 1.0, 5, 10 and 25 mg/l loadings. Up to 100% loss was observed in the 0.1 mg/l treatment in 24 hrs samples.

Analytical results — Measured BTEXN (mg/l)

		Nominal loading rate (mg/l)				
Day	Control	0.1	1.	5.0	10	25
0 (new)	ND	0.12	0.31	1.7	3.1	7.7
1 (old)	ND	0.12	0.41	1.6	3.3	7.1
1 (new)	ND	0.16	0.44	1.7	1.9	6.5
2 (old)	ND	0.15	0.45	1.6	2.1	6.8
2 (new)	ND	0.07	0.43	1.6	3.2	NA
3 (old)	ND	0.12	0.43	1.6	3.1	NA
3 (new)	ND	0.16	0.57	1.8	3.3	NA
4 (old)	ND	ND	0.56	1.8	3.5	NA

ND=not detected, NA=not analyzed due to 100% mortality

Guideline/protocol deviations: Body length smaller than recommended range of 4-6 cm; smaller fish used to minimize DO depletion in closed vessel (no-headspace) systems.
Test condition : Test solutions were prepared as water accommodated fractions

(WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.1, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the fish toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 20 liter stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish were approximately five weeks old at test initiation and were
obtained from Thomas Fish company, Anderson, CA, Lot 297. Loading of fish body mass to treatment was 0.3 g fish per liter of aqueous solution, mean length at termination was 3.3 cm (sd=0.2), and mean weight was 0.271 g (sd=0.064). Test vessels were 4 liter glass aspirator bottles with foil covered neoprene stoppers. Three replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEXN. Water temperature was 15 °C (0.1sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 609-614 Lux during full daylight periods. Dissolved oxygen measurements ranged from 5.4 to 9.7 ppm, pH values between 6.8 and 8.2.

Test substance : Gasoline Sample W94/814, Blend; Detailed hydrocarbon analysis:

N-paraffins:	16% total C_4-C_8
Iso-paraffins:	25% total C_4-C_{11}
Olefins:	12%, C_4-C_7
Naphthenes:	5% C_6-C_{10}
Aromatics:	42% C_6-C_{11}

Reliability : (1) valid without restriction
21.11.2001 (31)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : LL_{50} at 96 hr calculated using Probit procedure (Finney, D.J., 1971. Probit Analysis, Third Edition, London: Cambridge University Press, and SAS computer statistics software.
:

Result

Mortality (no. of deaths/treatment) at 96 hrs:

Treatment	No. of deaths
0	1
1.0	0
5	0
10	7
25	15
50	5

96-hr LL_{50} = 11 mg/l, 95% C.I: 8.7-16 mg/l (as nominal loading rate)
96-hour No Observed Effect Loading (NOEL) was 5 mg/l, both calculated (Dunnett's Procedure) and observed.
Results are quoted in terms of 50% Lethal Loading (LL_{50}), the loading rate of test substance resulting in 50% mortality of the test species exposed to the WAF. At termination, abnormal behavior/appearance (lethargy, erratic swimming) was observed in all surviving fish at the 10 mg/l treatment. Losses of the soluble components from the WAF over each 24 hour period ranged from 5 to 25% for the 5, 10 and 25 mg/l loadings. Up to 57% loss was observed in the 1.0 mg/l treatment in 24 hrs samples. BETXN concentrations on 24hour samples of the 50 mg/l treatments due to complete mortality on day 0 were not determined.

	Analytical results Measured BTEXN (mg/l) Nominal loading rate (mg/l)					
Day	Control	1.0	5.0	10	25	50
0 (new)	ND	0.54	2.3	4.2	9.5	20
1 (old)	ND	0.50	2.3	4.0	10	NA
1 (new)	ND	0.47	1.7	4.2	NA	NA
2 (old)	ND	0.20	2.1	4.0	NA	NA
2 (new)	ND	0.52	2.0	4.1	NA	NA
3 (old)	ND	0.25	2.0	4.3	NA	NA
3 (new)	ND	0.57	1.6	4.0	NA	NA
4 (old)	ND	0.38	1.2	3.2	NA	NA

ND=not detected, NA=not analyzed due to 100% mortality

Guideline/protocol deviations: Body length (2.7cm av.) smaller than recommended range of 4-6 cm; smaller fish used to minimize DO depletion in closed vessel (no-headspace) systems.

Test condition : Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 1, 5, 10, 25 and 50 mg/l were used to prepare test solutions for the fish toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 20 liter stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish

were approximately four weeks old at test initiation and were
obtained from Thomas Fish company, Anderson, CA, Lot 297. Loading of fish body mass to treatment was 0.2 g fish per liter of aqueous solution, mean length at termination was 2.7 cm (sd=0.2), and mean weight was 0.136 g (sd=0.034). Test vessels were 4 liter glass aspirator bottles with foil covered neoprene stoppers. Three replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEXN. Water temperature was 14.1 °C (0.03sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 619-622 Lux during full daylight periods. Dissolved oxygen measurements ranged from 7.4 to 9.8 ppm, pH values between 7.8 and 8.1.

Test substance : Gasoline Sample W94/813, Blend
Detailed hydrocarbon analysis:
N-paraffins: 20% total C_3-C_8,
Iso-paraffins: 28% total C_4-C_9
Olefins: 1%, C_5-C_7
Naphthenes: 5% C_5-C_{10}
Aromatics: 46% C_6-C_9

Reliability : (1) valid without restriction
21.11.2001 (12)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Acute immobilization test
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : EL_{50} calculated using the probit procedure (Finney, D.J., 1971. Probit Analysis, 3rd Ed. London: Cambridge Univ. Press)
Result : 48 hr results-number of organisms affected and analytical results

Treatment	Immobilization	Measured BTEXN -day 0	Measured BTEXN -day 2
Control	2	ND	ND
0.1 mg/l	1	0.12	0.20
1.0 mg/l	1	0.31	0.42
5.0 mg/l	1	1.7	1.4
10 mg/l	5	3.1	3.2
25 mg/l	20	7.7	7.1

based upon nominal loading rate 48-hr EL_{50} = 12 mg/l (95% C.I. 7.3 to 22 mg/l)

Test condition : Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend

water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.1, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 4 liter stoppered containers with less than 10% headspace volume. The WAF mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN on day 0 and at termination. Test vessels for daphnid testing were 125 ml glass erlenmeyer flasks with foil covered neoprene stoppers. Four replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. During the study test system solutions: dissolved oxygen concentration range: 7.2 to 9.2; pH ranged from 7.5 to 7.8; temperature was 19 °C (sd:0.2).Daphnia magna were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house.

Test substance : Gasoline Sample W94/814, Blend
Detailed hydrocarbon analysis:

N-paraffins:	16% total C4-C_8
Iso-paraffins:	25% total C_4-C_{11}
Olefins:	12% C_4-C_7
Naphthenes:	5% C_6-C_{10}
Aromatics:	42% C_6-C_{11}

Reliability : (2) valid with restrictions. Three previous attempts to conduct study were invalidated due to excessive (>20%) control mortality.

21.11.2001 (11)

Type : Acute immobilization test
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : EL_{50} calculated using the probit procedure (Finney, D.J., 1971. Probit Analysis, 3rd Ed. London: Cambridge Univ. Press)
Result : 48 hr results-number of organisms affected and analytical results

Treatment	Immobilization	Measured BTEXN-day 0	Measured BTEXN-day 2
Control	0	ND	ND
0.5 mg/l	0	0.29	0.10
1.0 mg/l	0	0.28	0.10
5.0 mg/l	3	2.3	1.7
10 mg/l	16	3.9	3.1

	25 mg/l	20	8.8	10

based upon nominal loading rate48-hr EL_{50} = 7.6 mg/l (95% C.I. 6.4 to 9.3 mg/l) 48-hr NOEL = 1.0 mg/l

Test condition : Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 4 liter stoppered containers with less than 10% headspace volume. The WAF mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN on day 0 and at termination. Test vessels for daphnid testing were 125 ml glass erlenmeyer flasks with foil covered glass stoppers. Four replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. During the study test system solutions: dissolved oxygen concentration range: 7.2 to 9.2; pH ranged from 7.5 to 7.8; temperature was 20 °C. Daphnia magna were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house.

Test substance : Gasoline Sample W94/813, Blend
Detailed hydrocarbon analysis:
N-paraffins: 20% total C_3-C_8
Iso-paraffins: 28% total C_4-C_9
Olefins: 1% C_5-C_7
Naphthenes: 5% C_5-C_{10}
Aromatics: 46% C_6-C_9

Reliability : (2) valid with restrictions. Three previous attempts to conduct study were invalidated due to excessive (>20%) control mortality.

21.11.2001 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : EL_{50} values were calculated using the inverse extrapolation method of Snedecor and Cochran, Statistical Methods, 8th Ed., 1989, Iowa State University Press/Ames. NOEL values

calculated using ANOVA (Duncan D.B., 1975, Biometrics, 31, 339-359)

Result : Percent inhibition:
72 hour EL_{50} for average growth rate=3.1 mg/l
(0.15 to >25 mg/l CI @95%)
72 hour EL_{50} for area under the growth curve=1.4 mg/l
(0 to 20 mg/l CI @95%)
96 hour EL_{50} for average growth rate=3.7 mg/l
(0.34 to >25 mg/l CI @95%)
96 hour EL_{50} for area under the growth curve=1.1 mg/l
(0 to 22 mg/l CI @95%)
72 hour NOEL for average growth rate=0.5 mg/l
72 hour NOEL for area under the growth curve =<0.5 mg/l
96 hour NOEL for average growth rate =1.0 mg/l
96 hour NOEL for area under the growth curve =<0.5 mg/l

Nominal (mg/l)	Average cell density (cells/ml)		% Inhibition Average growth rate		Area under growth curve	
	72hr	96hr	72hr	96 hr	72hr	96hr
Control	1.6E5	3.9 E5	0	0	0	0
0.5	1.0E5	2.8E5	6.2	3.4	36	33
1.0	6.8E4	2.6 E5	12	5.5	51	44
5.0	4.6E4	1.2E5	21	22	53	65
10	2.4E3	3.7E3	90	89	93	98
25	BMDL	BMDL	99	99	98	99

BMDL=below method detection limit

Analytical results

Nominal (mg/l)	Measured Concentration (mg/l as BTEXN)	
	Day0	Day 4
Control	none detected	none detected
0.5	0.12	0.30
1.0	0.58	0.67
5.0	2.4	0.65
10	5.2	4.72
5	12	9.6

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the algal toxicity tests. Test material was added volumetrically to 2.0 liters of sterilized algal nutrient media (enriched with 100 mg/l of sodium bicarbonate) in 2.0 liter aspirator bottles covered with aluminum foil. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks containing ten 4mm glass balls that were completely

filled (140 ml) with treatment solution, inoculated with algae and sealed with glass stoppers. Algal cells were obtained from 6 day old laboratory stock cultures maintained in nutrient enriched media, at 24 °C (±2°) C under continuous illumination of 4300(±10%) lux. Original algal cultures (Strain 1648) were provided by the Department of Botany, University of Texas. Cell density of the algal stock culture inoculum was determined prior to study initiation with a Turner filter-fluorometer. Fluorometer readings were converted to cell numbers using a regression formula developed through cell counts. Three replicates were prepared for each treatment level and six replicates were prepared as control systems. The initial algal concentration was approximately 1.0 x 10^3 cells/ml in each replicate chamber. All test replicates were placed on a shaker table at 150 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 4300 to 4400 Lux as measured daily using a Licor photometric sensor. A sample volume of 3.5 ml was taken daily for density determinations, and an equivalent volume of reserve 24 hour WAF was used to replenish the displaced sample volume. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN at day 0 and 96 hr termination. BTEXN total concentration at termination was at least 80% of the initial concentration for all treatments, with the exception of the 5.0 mg/l exposure, which showed a loss of 73%. This excessive loss compared to the other treatments was determined to be due to sampling technique. Test temperature was 23 °C (sd=0.08)C. The average pH was 7.5 at initiation; and ranged from 9.2 (control) to 7.8 (25 mg/l loading) at termination.

Test substance : Gasoline Sample W94/813, Blend
Detailed hydrocarbon analysis:
N-paraffins: 20% total C_3-C_8
Iso-paraffins: 28% total C_4-C_9
Olefins: 1% C_5-C_7
Naphthenes: 5% C_5-C_{10}
Aromatics: 46% C_6-C_9

Reliability : (1) valid without restriction
21.11.2001 (8)

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995
GLP : yes
Test substance : Gasoline CAS No. 86290-81-5
Method : EL_{50} values were calculated using the inverse extrapolation method of Snedecor and Cochran, Statistical Methods, 8th Ed., 1989, Iowa State University Press/Ames. NOEL values calculated using ANOVA (Duncan D.B., 1975, Biometrics, 31, 339-359).
Result : Percent inhibition:
72 hour EL_{50} for average growth rate=3.3 mg/l
(0.24 to >25 mg/l CI @95%)
72 hour EL_{50} for area under the growth curve=4.2 mg/l
(0 to 24 mg/l CI @95%)
96 hour EL_{50} for average growth rate=2.5 mg/l
(0.62 to 14 mg/l CI @95%)

96 hour EL_{50} for area under the growth curve=0.25 mg/l
(0 to 26 mg/lL CI @95%)
72 hour NOEL for average growth rate and area under the growth curve =0.5 mg/l
96 hour NOEL for average growth rate = 0.5 mg/l
96 hour NOEL for area under the growth curve =<0.5 mg/l

Nominal (mg/l)	Avg cell density (cells/ml)		% Inhibition Avg growth rate		area under growth curve	
	72hr	96hr	72hr	96 hr	72hr	96 hr
Control	9.9E4	3.8 E5	0	0	0	0
0.5	7.7E4	2.6 E5	7.7	7.8	21	27
1.0	5.5E4	1.7 E5	15	17	36	50
5.0	2.5E4	2.2 E4	33	51	54	81
10	3.7E3	2.0 E3	76	95	90	97
25	BMDL	BMDL	99	100	98	99

BMDL=below method detection limit

Analytical results
Nominal (mg/l) Measured Concentration (mg/l as BTEXN)

	Day 0	Day 4
Control	none detected	none detected
0.5	0.22	0.23
1.0	0.47	0.51
5.0	1.5	1.3
10	3.5	3.3
25	9.5	7.7

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the algal toxicity tests. Test material was added volumetrically to 2.0 liters of sterilized algal nutrient media (enriched with 100 mg/l of sodium bicarbonate) in 2.0 liter aspirator bottles covered with aluminum foil. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks containing ten 4mm glass balls that were completely filled (140 ml) with treatment solution, inoculated with algae and sealed with glass stoppers. Algal cells were obtained from 5 day old laboratory stock cultures maintained in nutrient enriched media, at 24 °C (±2°) under continuous illumination of 4300(±10%) lux. Original algal cultures (Strain 1648) were provided by the Department of Botany, University of Texas. Cell density of the algal stock culture inoculum was determined prior to study initiation with a Turner filter-fluorometer. Fluorometer readings were converted to cell numbers using a regression formula

developed through cell counts. Three replicates were prepared for each treatment level and six replicates were prepared as control systems. The initial algal concentration was approximately 1.0 x 103 cells/ml in each replicate chamber. All test replicates were placed on a shaker table at 150 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 4300 to 4400 Lux as measured daily using a Licor photometric sensor. A sample volume of 3.5 ml was taken daily for density determinations, and an equivalent volume of reserve 24 hour WAF was used to replenish the displaced sample volume. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN at day 0 and 96 hr termination. BTEXN total concentration at termination was at least 80% of the initial concentration. Test temperature was 23 °C (sd=0.08). The average pH was 7.6 at initiation; and ranged from 9.5 (control) to 8.1 (25 mg/l loading) at termination.

Test substance : Gasoline Sample W94/814, Blend
Detailed hydrocarbon analysis:
N-paraffins: 16% total C_4-C_8
Iso-paraffins: 25% total C_4-C_{11}
Olefins: 12% C_4-C_7
Naphthenes: 5% C_6-C_{10}
Aromatics: 42% C_6-C_{11}

Reliability : (1) valid without restriction
21.11.2001 (9)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	undiluted
Value	:	= 18.75 ml/kg bw
Year	:	1980
GLP	:	yes
Test substance	:	API PS-6
Method	:	Groups of 10 fasted rats (five male and five female) were given API PS-6 at doses of 10, 15, 17.5, 20 and 25 ml/kg as a single oral dose. The animals were then allowed food and water ad libitum and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded. [In addition 2 extra males and one female were given 15 ml/kg because 3 of the original animals died soon after dosing and this was believed to be due to dosing injury. However, at necropsy, no evidence of injury was found and therefore all animals were included in the calculations for an LD50]
Result	:	Toxic signs were the same in all dose groups, increasing in severity with increasing dose. There were oily urine stains, but most of the test material seemed to be excreted via the feces. The area around the anus became very irritated. Diarrhea was common in each dose level and blood was commonly seen around the eyes, nose and mouth. Observations at necropsy were similar for all dose groups. Animals surviving to 14 days had very few abnormalities and these were usually of a minor nature such as enlarged Peyer's patches on the intestines. There were numerous instances of lung involvement in both surviving animals and those dying before 14 days. These changes consisted of mild irritation and congestion, to fluid filled abscesses. Almost all animals that died before 14 days had intestinal damage. The intestines, and often the stomach, became hemorrhagic and sometimes blood was observed in the intestine or stomach. The intestine wall became thin and there was an increased amount of gas in the gastro intestinal tract. The heart was enlarged or irregularly shaped in some rats. Mortality and body weight changes are summarized in the following table.

Dose group (ml/kg)	Mortality dying/dosed	Weight change (over 14days)
Males		
10	0/5	weight gain
15	2/7	weight loss
17.5	3/5	weight loss
20	4/5	weight loss
25	5/5	weight loss

Females		
10	0/5	weight gain
15	1/6	weight gain
17.5	3/5	weight loss
20	0/5	weight gain*
25	4/5	weight loss

* one animal had a weight loss over the 14 day period.

The oral LD_{50} was determined to be 18.75 ml/kg. The 95% confidence limits were 16.3 to 21.6 ml/kg.

Reliability : (1) valid without restriction
19.11.2001 (5)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 4
Vehicle : undiluted
Value : > 5 ml/kg bw
Year : 1979
GLP : yes
Test substance : API PS-6
Method : The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin.
A single dose of undiluted test material (5 ml/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later and the animals were observed for a total of 14 days post-dosing.
At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Result : When the patches were removed following dosing dark red to almost purple skin was seen in all animals. Slight erythema and dry skin was observed in all rabbits during the study.
With the exception of one animal all animals weighed more at the end of the study than they did at study commencement.
One female rabbit died on day 6 of the 14 day study and the gross necropsy revealed slightly congested lungs, no food in the stomach and white areas in the liver.
At necropsy of the surviving animals four rabbits had congested lungs, one had pale kidneys, one had an irritated stomach lining and one had enlarged Peyer's patches on the jejunum. These observations were considered to be normal and not dose-related.
Reliability : (1) valid without restriction
19.11.2001 (5)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Remark : Not appropriate
04.07.2001

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : .98
Result : slightly irritating

Method : Draize Test
Year : 1979
GLP : yes
Test substance : API PS-6
Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each of 3 male and 3 female rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing.
After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.
Result : A summary of the dermal irritation scores is given below.

	Exposure time (hours)	Average value of all animals
Erythema		
Intact skin	24	0
	72	0.92
Abraded skin	24	0
	72	1.0
Edema		
Intact skin	24	0.5
	72	0.5
Abraded skin	24	0.5
	72	0.5
		3.92
Primary irritation score =Total÷4=		0.98

Edema but no erythema was noted at 24 hours, although the test area was whiter than the surrounding skin.
At 72 hours erythema and edema were observed.
By 7 days almost all erythema had cleared but some edema was still present and the test site was dry and flaky.
By day 14 all edema and erythema had cleared but there was no hair growth at this time.

Reliability : (1) valid without restriction
19.11.2001 (5)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure Time	:	See method
Comment	:	rinsed after (see exposure time)
Number of animals	:	9
Result	:	not irritating
Method	:	Draize Test
Year	:	1979
GLP	:	yes
Test substance	:	API PS-6
Method	:	0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits (4 male, 5 female), the other eye was untreated and served as control. After 20 to 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.
Result	:	No irritation was observed in any animal at any of the three observation times. Animals whose eyes had been irrigated following instillation of test material were no different from those whose eyes had not been washed.
Reliability	:	(1) valid without restriction

19.11.2001 (5)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	guinea pig
Concentration	:	Induction 50 % occlusive epicutaneous Challenge 50 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	mineral oil
Result	:	not sensitizing
Year	:	1979
GLP	:	yes
Test substance	:	API PS-6
Method	:	0.5 ml of undiluted test material was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residues of test material. After the first application, irritation was sufficiently severe that for further dosing a 50% dilution in mineral oil was used. The animals received one application 3 times each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.5 ml of a 50% dilution in mineral oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the

test site was depilated 3 hours prior to reading by using a commercially available depilatory cream.

Positive control (0.05% 2,4-dinitrochlorobenzene in ethanol), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups.

Result : On a subjective basis, the challenge treatment did not appear to be more reactive than the sensitizing treatments. The average scores for erythema and edema following induction
and challenge are summarized below.

Average scores	PS-6 gasoline Erythema	Edema	Positive control Erythema	Edema
Induction	0.9	0.3	1.3	0.3
Challenge	0.1	0	1.9	1.7

The authors concluded that the test material was not sensitizing.

Reliability : (2) valid with restrictions
Although the study was conducted to GLP, there was no vehicle control and the results from the positive control were not convincing.

21.11.2001 (5)

5.4 REPEATED DOSE TOXICITY

Species : Rats and monkeys
Sex : male/female
Strain : Sprague Dawley rats and squirrel monkeys
Route of admin. : inhalation
Exposure period : 6 hours each day
Frequency of treatment : 5 days a week for 13 weeks
Post obs. period :
Doses : leaded gasoline: 0, 100 & 400 ppm. Unleaded gasoline: 0, 400 & 1500 ppm
Control group : yes, concurrent no treatment
Year : 1984
GLP : no data
Test substance : leaded and unleaded gasoline
Method : This study was conducted as a preliminary range finding study prior to conducting a two year study on the same test materials.

20 rats and 4 monkeys of each sex were housed in 1m³ glass and stainless steel exposure chambers 24 hours a day and were only removed for cleaning purposes. Target exposure vapor concentrations of the test materials were:
Unleaded gasoline: 400 and 1500 ppm
Leaded gasoline: 100 and 400 ppm
A control group of 20 rats and 4 monkeys of each sex were exposed to air only.
Exposures were for 6 hours each day, 5 days each week for 13 weeks.

Blood was taken from 10 rats of each sex at the end of the study from the highest dose groups only for hematological evaluation. Blood was taken from all monkeys in the highest dose group at 1.5, and 3 months.

Urine samples were analyzed for all animals at 1.5 and 3 months for levels of protein, glucose, ketones, bilirubin, blood and lead.

CNS evaluations were conducted on the monkeys in the control and high level dose groups at before exposure and at 3 months. The CNS evaluations consisted of recording simultaneous and evoked responses and this was accomplished using electrodes that had been implanted permanently in the visual cortex.
Pulmonary function tests similar to those reported by Alarie were conducted on all monkeys prior to exposure and at 1.5 and 3 months on the control and high level unleaded groups.
All animals that died or were sacrificed at termination of the study were subjected to a gross necropsy. Organ weights were recorded and lungs, kidneys, spleen, heart, brain and bone marrow from the control and high dose groups were evaluated for histopathology.
All male and female animals from the control and high exposure groups were also evaluated for the presence of IgG in the renal glomerulus and lungs.
A lead analysis was also made on rat brain, kidney, liver, urine and blood from both the leaded dose groups and controls.

Result : The mean exposure concentrations were found to be as follows:

	Gasoline vapor exposure concentration		
Group	Mg/l ±SD	ppm	Alkyl lead µg Pb/l ±SD
Control	-	-	-
Unleaded 1500 ppm	6.35±0.44	1552	-
Unleaded 400 ppm	1.57±0.15	384	-
Leaded 400 ppm	1.53±0.23	374	0.72±0.1
Leaded 100 ppm	0.42±0.04	103	0.19±0.04

Three rats at different dose levels and three monkeys also at different dose levels died during the study. These deaths were not considered to be treatment-related.
Two female monkeys in each of the high dose groups exhibited emesis, 13 and 17 days after commencing exposure for the 1500 ppm unleaded and 400 ppm leaded groups respectively.
Although there was a reduction in body weights in males in the lowest dose group of each of the test materials but by the end of the study they were demonstrating increased weights. No differences were observed in any of the other treated groups.
The hematological values for the monkeys exposed to either test material at either dose level were similar to those fo the control animals. In the rats the only changes observed were:

unleaded (1500 ppm males)	64% increase in thrombocytes
unleaded (1500 ppm females)	150% increase in reticulocytes
leaded (400 ppm males)	4% decrease in MCHC
leaded (400 ppm females)	10% increase in hematocrit
leaded (400 ppm females)	11% increase in MCV
leaded (400 ppm females)	decrease in WBC

Mean flash-evoked response time for the monkeys was measured prior to exposure and was unaffected by exposure.

The results of the mean pulmonary function data are summarised in the following table. Only increases (% I) or decreases (% D) compared to controls are shown in the table. All other parameters were similar for treated and control animals.

	Pre-exposure	42 days	90 days
Respiratory rate			
Unleaded 1500 ppm F	-	-	-
Unleaded 1500 ppm M	30% D	21% D	-
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	-
Tidal volume			
Unleaded 1500 ppm F	-	-	22% D
Unleaded 1500 ppm M	-	-	-
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	-
Minute volume			
Unleaded 1500 ppm F	-	-	-
Unleaded 1500 ppm M	-	-	36% I
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	53% I

There were no effects on airway resistance, dynamic compliance or breaths to 1% nitrogen.

Urinalysis showed no differences between treated and control animals in either species.
There was no evidence of IgG deposition in the kidneys of rats or monkeys of either sex following exposure to the test materials for 90 days.
Group mean lead levels in the rat tissues were as follows:

		Control	Leaded 400 ppm	Unleaded 100 ppm
Brain	M	1.26	9.49	7.23
	F	1.44	5.39	2.32
Kidney	M	1.71	12.4	7.06
	F	2.97	9.57	13
Liver	M	0.71	17.9	6.51
	F	1.21	19.7	8.41
Blood	M	0.61	6.1	0.77
	F	0.24	1.32	0.46
Urine	M	0.17	0.21	0.19
	F	0.31	0.18	0.25

No actual values are given on organ weights or organ/body weight ratios but the following effects are reported:

Rats

	Liver wt	Kidney wt
Unleaded 400 ppm M	increased	
Unleaded 400 ppm F		
Unleaded 1500 ppm M		
Unleaded 1500 ppm F		
Leaded 400 ppm M		
Leaded 400 ppm F	decreased	
Leaded 100 ppm M	increased	
Leaded 100 ppm F		increased

Monkeys

	Thyroid	Kidney
Unleaded 400 ppm M	increased	
Unleaded 400 ppm F		
Unleaded 1500 ppm M	increased	
Unleaded 1500 ppm F		
Leaded 400 ppm M		decreased
Leaded 400 ppm F		
Leaded 100 ppm M		
Leaded 100 ppm F		

Organ weights were also expressed as % of body weight and the following effects were recorded:
Rats:
Decreased heart weight in both male leaded groups
Decreased brain weight in both male unleaded groups
Decreased liver weight in 400 ppm female leaded group
Decreased adrenal weight in 1500 ppm female unleaded group.
Monkeys:
Decreased kidney weight in 400 ppm male unleaded group.

No evidence of treatment-related histopathology was observed in either rats or monkeys, with the exception of lesions noted in the kidneys of all male rats. The lesions were characterized by subtle but discernible increases in the incidence and severity of regenerative epithelium and dilated tubules. The latter were seen to contain protein in their lumens.

Test substance : An unleaded EPA reference fuel and a commercially available leaded gasoline were tested. The compositional properties of the two fuels were as follows:

	Unleaded fuel	Leaded fuel
Calculated data		
Research octane No.	93	87
Motor octane No.	88	86
Reid vapor pressure (PSIA	6.9	6.3
Distillation °F (ASTM D-86)		
Initial boiling point	80	80
10%	135	160
50%	210	217
90%	275	295
100%	345	340
FIA analysis		
% aromatics	30.1	27.4
% olefins	8.2	7.8
% saturates	61.7	64.8
Experimental data		
API gravity at 60°F	57.0	58.4
Sulfur, ppm	240	75
Lead, g/gallon	<0.005	1.94
Benzene, LV%	0.2	0.4
Toluene, LV%	16.7	11.4
n-Butane, LV%	1.0	0.4
Isopentane, LV%	5.4	5.5
n-Pentane, LV%	4.8	4.0

Reliability : (2) valid with restrictions
19.11.2001

(20)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Microbial mutation assay in Salmonella typhimurium and Saccharomyces cerevisiae
Metabolic activation : with and without
Result : Negative
Year : 1977
GLP : no data
Test substance : Unleaded gasoline
Method :

The solubility, toxicity and dose levels for the test material were determined prior to the mutagenicity screening.
DMSO was used as solvent.
Based on the preliminary studies the following concentrations of test material were used in the mutagenicity assays:

Test doses	% Concentration	
	Bacteria	Yeast
1/8 50% survival	0.375	0.625
1/4 50% survival	0.75	1.25
1/2 50% survival	1.5	2.5
50% survival	3	5

Plate tests
For non-activation assays cells in broth were exposed to the test material at the concentrations shown above. The contents of the tubes of broth plus test material were poured over selective agar plates which were then incubated. The test was conducted with and without metabolic activation.
Positive control substances (see results section) were also run in the same assay.

The following evaluation criteria were used in this plate test.

Strains TA1535, 1537 and 1538
If the solvent control value is within the normal range a chemical which produces a positive response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

Strains TA98, 100 and D4
If the solvent control value is within the normal range, a chemical which produces a positive response over three concentrations with the highest increase equal to twice the solvent control value for TA100 and two to three times the solvent control value for strains TA98 andD4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

Pattern
Because TA1535 and TA100 were both derived from the same parental strain (G-46) and because TA1538 and TA98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the

two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain responds to a mutagen in non-activation tests it will generally do so in activation tests, but the converse of this is not anticipated.
While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

Reproducibility
If a chemical produces a response in a single test which cannot be repeated in one or more additional runs, the initial positive test data loses significance.

The above criteria are not absolute and other extenuating factors may enter into a final evaluation decision.

Suspension tests
Bacteria and yeast cultures were grown in complete broth. The cells were removed, washed and exposed to the test material at the concentrations shown in the results section. For the yeast cells exposure to the test material was for 4 hours whereas for the bacterial cells exposure was for 1 hour. Aliquots of the cells were plated onto the appropriate complete media. After suitable incubation periods, the number of revertant colonies were counted. This assay was also conducted with and without metabolic activation and positive control substances were also included.

The following criteria were used in the suspension assay.

Surviving population counts
A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests show very low (<25%) survival compared to the tissue controls. Data of this
type are generally unacceptable and these experiments are repeated at a lower dose level.

Total mutant counts
For non mutagens, the ratio of mutant to surviving population
should be roughly equivalent for each test point in a given experiment.
A mutagenic chemical will produce an altered mutant/surviving population ratio. An attempt is made to keep the surviving population of cells high and to look for positive responses that show increases in both numbers of mutants and mutation frequencies.

Dose-response
Dose-related increases in mutants and mutation frequencies are the most convincing data when assessing mutagenic activity. To ensure a proper dose response, dose levels are kept within a relatively low range o

Result : Plate test
There was no increase in revertants caused by exposure to the test material at any concentration. The results in this assay were negative both with and without metabolic activation.

Suspension test

The mutation frequencies are summarized in the following table for assays with and without metabolic activation.

Non activation assay

Dose level	Salmonella strains TA100	TA1535	TA1537	TA1538	TA98*	Yeast D4**
-ve control	5.48	3.59	6.15	7.1	41.99	23.69
+ve control	125.51	185.65	161.54	84.75	100	66.29
1 (low)	18.18	2.26	12.54	27.78	233.33	9.52
2	2.9	2.15	8.97	11.76	63.04	36.99
3	3.1	2.98	7.19	10	9.56	30.02
4 (high)	4.13	2.66	9.68	3.21	35.74	32.38

* Assay repeated for negative control and lowest 2 doses.
Results were 54.59 for -ve control
10.84 for lowest dose
14.11 for next highest dose

** Assay repeated at all dose levels
Results were: -ve control 4.66
+ve control 97.73
dose level 1 1.3
dose level 2 8.33
dose level 4 12.65

Slight increases are observed at the high dose levels with TA100, TA1537 and TA1538. However the responses are not adequate enough to be considered positive. The increases with TA98 could not be reproduced.

With activation

Dose level	Salmonella strains TA100	TA1535	TA1537	TA1538	TA98*	Yeast D4**
-ve controls*						
A+C	17.08	5.25	6.01	4.8	21.01	52.66
A-C	17.29	8.77	9.29	8.25	62.02	7.96
AL1	17.34	7.32	3.99	6.48	45.03	30.06
+ve control	25.51	89.92	0.22	1253.4	555.35	115.3
1 (low)	22.97	41.67	100	71.43	100	
2	15.64	7.21	0	300	30.66	27.22
3	17.26	9.57	20	15.38	83.33	27.03
4	22.31	7.21	5.43	6.93	60.13	29.04

* Controls were
A+C No activation system but including positive control
A-C Solvent control, no test chemical or activation system
AL1 Liver homogenate control plus solvent

Scattered increases were found at one or more dose levels (see table above). All apparent positive effects were repeated and were not reproducible indicating problems associated with the initial runs. When the raw data were inspected it was observed that the increases were due to anomalous reductions in viable cell counts. The results of this assay were therefore considered to be negative.

Reliability : (2) valid with restrictions
Valid with restrictions due to poor quality of initial assay.

19.11.2001 (2)

Type	:	Mouse lymphoma assay
System of testing	:	Forward mutation assay using cell line L5178Y TK+/-
Metabolic activation	:	with and without
Result	:	negative
Year	:	1977
GLP	:	no data
Test substance	:	Unleaded gasoline
Method	:	The test material was dissolved in acetone for this assay. The positive control substances were Ethyl methane sulphonate (EMS) and Dimethylnitrosamine (DMN). A cytotoxicity study was carried out prior to the mutagenicity assay. For the mutation assay the lymphoma cells were exposed for 5 hours to test material at concentrations ranging from 0.065 to 1.04 µl/ml for both the activation and non-activation assays. After exposure to the test material, the cells were allowed to recover for 3 days and then cultures were selected for cloning and mutant selection. Surviving cell populations were determined by plating diluted aliquots in non-selective growth medium. A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. A compound is considered mutagenic if: A dose response relationship is observed over 3 of the 4 dose levels employed. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value. The solvent control data are within the normal range of the spontaneous background for the TK locus.
Result	:	Little toxicity was observed with the test material. Positive control values exhibited significant responses over the negative controls, and the negative controls were within the normal range. All results for the test material from the non-activation assay were negative. The results from the activation assay were also considered to be negative. There was an increase in the number of mutants at the 0.52 µl/ml concentration but this appeared to result from a slight increase in the number of viable clones. There was no trend indicating a dose-related response and, therefore, the increases were not believed to be compound related.

The results are summarized below.

Dose (µl/ml)	Rel. susp. growth	Mutant clones	Viable clones	% Rel. growth	Mutant frequency	
Non-activation						
0.065	121.8	76	159	139.3	0.478	
0.13	103.7	29	215	160.4	0.1349	
0.26	114.6	44	211	174	0.2085	
0.52	141.8	66	161	164.3	0.4099	
1.04	107.5	58	270	208.9	0.2148	
Solvent		100	14	139	100	0.1007
Negative	129.9	41	140	130.8	0.2929	
EMS	58.7	227	67	28.3	3.3881	
Activation						
0.065	120.6	66	87	79.5	0.7586	
0.13	108.6	46	126	103.7	0.3651	
0.26	106	70	130	104.4	0.5385	
0.52	112.4	92	108	92	0.8519	
1.04	68.9	21	193	100.8	0.1088	
Solvent		100	30	132	100	0.2273
Negative	92.1	41	150	104.7	0.2733	
DMN	16.7	91	7	0.9	13	

Reliability : (1) valid without restriction
19.11.2001 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex : male
Route of admin. : i.p.
Doses : Acute study: 0.024, 0.08 & 0.24 ml/rat; subacute study: 0.01, 0.03 & 0.1 ml/rat
Result : negative
Year : 1977
GLP : no data
Test substance : API PS-6 unleaded gasoline
Method : Two studies were conducted viz an acute and a subacute study. The test material was administered to the animals intraperitoneally in acetone.

Acute study
Groups of 15 rats were given either acetone (0.1 ml/rat), or test material at doses of 0.024, 0.80 or 0.24 ml/rat. An additional group of 5 rats were given Triethylenemelamine(TEM)at a dose of 0.3 mg/kg.
6, 24 and 48 hours after administration of the test material 5 animals in each dose group were killed. For the TEM group, all five animals were killed 24 hours after administration of the substance.
Two hours prior to being killed , cells were arrested in metaphase by the administration of a single i.p. dose of colchicine (4 mg/kg).
Bone marrow was aspirated from the femurs and tibias of the lower limbs of the animals after they had been killed. The marrow plug was washed and then fixed. Slides of the cells were prepared and stained with Giemsa for microscopic examination. Fifty spreads were located for each animal and when of suitable quality, the chromosomes were counted and

evaluated for the presence of abnormalities.

Subchronic study
18 animals were used in this study. They were dosed with three levels of test compound (0.01, 0.03 & 0.1 ml/rat) once each day for 5 days. All animals were killed 6 hours after administration of the last dose and 2 hours prior to being killed they were treated with colchicine in the same way as the animals in the acute study.
Slides were prepared and examined as for the acute study. A negative and positive control group were also included, again the same as for the acute study.

Remark : A subsequent study (API ref 26-60099) was also carried out and this supported the negative conclusion of the original study. The second study is not summarized here.

Result : The results of the acute and repeat dose studies are summarized in the following table:

Material & dose (ml/rat)	Time after dose	No of rats	Total No of cells	% cells with aber*	Mitotic index
Acute study					
Acetone (0.1)	6	3	100	1	3.4
	24	3	100	0	4.3
	48	3	150	0	3.8
TEM (0.3 mg/kg)	24	5	200	30	3.8
PS-6 (0.024)	6	5	250	6	3.9
	24	5	250	1	4.9
	48	5	250	3	4.7
(0.08)	6	5	250	1	5.6
	24	5	200	3	4.7
	48	5	100	5	2.7
(0.24)	6	5	187	2	3.2
	24	3	100	0	4.1
	48	5	200	3	4.5
Subacute study					
Acetone (0.1)	5 days	3	150	1	5.8
PS-6 (0.01)	5 days	5	200	1	3.5
(0.03)	5 days	4	159	2	2.9
(0.1)	5 days	5	174	2	3.2

* = aberrations

The results of the acute study were considered to be negative. There was an increase in aberrations at the 48 hour sacrifice period of the intermediate dose. The increases of 5% was significant, but did not fit into a trend suggestive of a compound-related mutagenic response. No other increases were observed at any dose level or sacrifice time.

The results of the subacute study were considered to be negative. There was no indication of an increased number of cells with aberrations.

Reliability : (1) valid without restriction
19.11.2001 (2) (3)

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	inhalation
Exposure period	:	6 hours/day, 5 days/week for 8 weeks
Doses	:	400 &1600 ppm
Result	:	negative
Year	:	1980
GLP	:	yes
Test substance	:	API PS-6 unleaded gasoline
Method	:	Groups of 10 male mice were exposed to either filtered air (negative controls) or test material at concentrations of 400 or 1600 ppm. Generation of test atmospheres was accomplished by bubbling air through the test material. Exposures were for 6 hours a day, 5 days each week for 8 weeks. On the final day of exposure a positive control group of 10 male mice were given Triethylenemelamine (TEM) intraperitoneally as a single i.p. dose, at a dose level of 0.3 mg/kg. The dose volume was 0.1 ml/mouse and the TEM was dissolved in 0.9% saline. Chamber concentrations were monitored at least hourly during the exposure periods. After 2 days rest following termination of exposures, each male was caged with 2 unexposed virgin female mice. At the end of 5 days, the females were removed. This weekly mating sequence was continued for 2 weeks. Each pair of mated females were transferred to a fresh cage and after 14 days after the midweek of being caged with the male were sacrificed. The uterine contents of the females were examined and scored for the numbers of dead and living implants and total implants. <u>Evaluation Criteria</u> Dominant lethality was determined from a) a mutation index derived from the ratio of total to dead implants; or b) the number of dead implants per pregnant female. If true dominant lethality is observed then a significant increase in the number of dead implants per pregnant female should be accompanied by a significant decrease in the number of living implants per pregnant female. The two ratios are compared with both concurrent and comparable historical control values. Dose-related trends are also looked for. Any statistically significant differences must also be strongly evaluated for their biological significance. In this study the following parameters were determined: Fertility index ie. Proportion of pregnant females. Average No of implants/pregnant female. Average No. of dead implants/pregnant female. Proportion of females with one or more dead implants. Proportion of females with two or more dead implants.
Result	:	During the exposure phase actual chamber concentrations were found to be 0, 396.4 and 1524.6 ppm. One male died in the 1600 ppm group and another animal in the same group exhibited excessive lacrimation in the seventh

week but this cleared in the final week.

The data for each of the parameters determined are as follows for untreated control, historical control, positive control and the two groups exposed to test material.

Week	Hist. -ve	-ve	+ve	400 ppm	1600 ppm
Fertility index					
1	22/24	21/23	19/24	17/20	21/22
2	16/24	19/24	13/24	18/19	16/22
Av. No. of implants/pregnant female					
1	267/22	240/21	140/19	203/17	214/21
2	193/16	220/19	91/13	219/18	183/16
Av. No. of dead implants/pregnant female					
1	12/22	14/21	83/19	9/17	9/21
2	13/16	5/19	66/13	9/18	12/16
Proportion of females with one or more dead implants					
1	11/22	9/21	19/19	6/17	8/21
2	9/16	4/19	13/13	8/18	7/16
Proportion of females with two or more dead implants					
1	1/22	3/21	17/19	3/17	1/21
2	2/16	1/19	13/13	1/18	3/16
No of dead implants/total implants					
1	12/267	14/240	83/140	9/203	9/214
2	13/193	5/220	66/91	9/219	12/183

Interpretation of the results:
The test material did not cause any significant reduction i the fertility index.
The test material had no effect on the average number of implants per pregnant female.
With respect to the number of dead implants per pregnant female, the test material showed no significant differences from the values of the concurrent as well as the negative controls.
The results support the conclusion that the test material did not cause increases in post-implantation deaths.

Reliability : (1) valid without restriction
19.11.2001 (6)

5.7 CARCINOGENITY

Species	:	rat and mouse
Sex	:	male/female
Strain	:	F 344 rat B6C3F mouse
Route of admin.	:	inhalation
Exposure period	:	Up to 113 weeks
Frequency of treatment	:	6 hours/day, 5 days/week
Doses	:	50, 275 & 1500 ppm nominal concentration
Control group	:	yes
Method	:	Similar to NCI guidelines
Year	:	1984
GLP	:	yes
Test substance	:	API PS-6 gasoline
Method	:	Groups of 100 rats of each sex and 100 mice of each sex were exposed to wholly vaporized gasoline at nominal concentrations of 50, 275 and 1500 ppm. 100 mice and 100 rats of each sex were exposed to air only and served as controls. Whole body exposures were in 16 m3 glass and stainless steel chambers. Exposures were for 6 hours a day, 5 days each week for up to 113 weeks. All animals were individually housed and were allowed free access to food and water except during the exposure periods. Any animals that died during the first 10 days of exposure were replaced but thereafter no replacements were made. All animals were observed twice daily, once before and once after the exposure period. Animals found moribund were removed from the study and sacrificed. All animals were examined once per month for clinical signs and palpable tissue masses. Body weights were recorded monthly for the first 17 months and bi-weekly thereafter. After approximately 18 and 24 months exposure 7 male and 7 female rats from each dose group were selected and hematological and clinical evaluations were conducted on these. After 3, 6, 12 and 18 months exposure 10 rats and 10 mice of each sex from each dose group were sacrificed and underwent complete post mortem examinations. At study termination all surviving animals were sacrificed. Body weight were recorded and after gross examination a wide range of organs/tissues were removed, weighed and fixed for subsequent histopathological examination.
Result	:	Monitoring of the exposure chamber concentrations established that actual concentrations for the study were: 0, 67, 292 and 2056 ppm. <u>Results of study in rats</u> There were very few pharmacotoxic signs that occurred in only a few animals and insufficiently frequently to be considered treatment-related. Mortality rates were also unaffected by exposure to gasoline vapor. Male rats in the highest dose group had lower body weights than controls from week 5 throughout the study. The difference amounted to 33 g at week 44 and this remained throughout. Females at the highest dose also weighed less than controls. A difference of 30 g had occurred by week 66 and this remained throughout the study.

The few differences in hematological data between controls and several treatment groups were within the normal range for rats of similar age and not considered to be treatment-related.

Similarly, small changes in a few clinical chemical parameters were not considered to be treatment-related.

At gross necropsy at the 3, 6 12 and 18 month sacrifice the only significant macroscopic findings were in the kidneys of the high dose group male rats and these consisted of tan color, foci, mottling, discolored and granular surface. Although the incidence was small it was considered significant in the light of the histopathology findings. Additionally, masses or nodules were observed in mid (3 masses/nodules) and high (5 masses/nodules) dose male kidneys that died between 18 months and study termination. There were no other gross findings.

Microscopic pathology examination revealed an increase in the incidence of renal disease with tubular degeneration and regeneration or cystic dilatation in the mid and high dose males from 3 months onwards. At 24 months primary renal neoplasms were observed in the following incidence:

Dose group	Neoplasm	Males	Females
0 ppm		0	0
50 ppm	renal carcinoma	1	0
275 ppm	renal adenoma	2	0
	renal carcinoma	2	0
	renal sarcoma	1	1
2056 ppm	renal carcinoma	6	0
	renal adenoma	1*	

* Occurred at 18 months

With the exception of one renal sarcoma all other tumors occurred in males.

Results of study in mice

There were no consistent signs of toxicity attributable to treatment and mortality rates were considered to be unaffected by treatment. Growth rates were similar for treated and control groups up until approximately week 70 after which the highest dose group males and females had lower body weights than controls. The difference amounted to approximately 2.3 to 4.4g on a body weight of 35g for males and 2 to 3g on a weight of 33g for females. The investigators considered this reduced body weight to be attributable to treatment. Organ weights were unaffected by treatment.
There was an increased incidence of liver nodules and masses in treated females in the high dose group that died on the study from 18 months to termination and which were terminally sacrificed. The incidence is tabulated as follows:

	Dose group (ppm)			
	0	67	292	2056
MALES				
Dead on study plus 18mth group	3/5	2/7	3/14	4/8
Terminal sacrifice	14/46	12/35	13/30	21/46
Total	17/51	14/42	16/44	25/54

FEMALES

Dead on study				
plus 18mth group	4/16	4/19	7/20	8/14
Terminal sacrifice	5/41	6/33	8/37	18/42
Total	9/57	10/52	15/57	26/56

There was a possible reduction in the incidence of cystic or enlarged uteri for female mice.
The incidences were

0 ppm	38/41
67ppm	26/33
292ppm	19/37
2056ppm	12/42

There were no other treatment-related findings at necropsy.

Microscopic examination of the tissues of animals up to and including the 18 month sacrifice did not reveal any compound-related effects.

At 24 months, however, there was an increased incidence of hepatocellular tumors in the high dose group females when compared to controls. The actual incidence of liver tumors is shown in the following table.

	Dose group (ppm)			
	0	67	292	2056
Number examined	57	52	57	56
Hepatocellular adenoma	1	4	4	8
Hepatocellular csarcinoma	7	6	9	20
Animals with hepatocellular tumors*	8	10	12	27

* Some animals had more than 1 tumor.

No other compound-related lesions were observed.

11.12.2001 (18) (19) (21)

5.8 TOXICITY TO REPRODUCTION

Type	:	Two generation study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hours
Frequency of treatment	:	Daily
Premating exposure period		
Male	:	10 weeks
Female	:	10 weeks
Duration of test	:	Two generations
Doses	:	5000, 10 000 &20 000 mg/m^3
Control group	:	yes
NOAEL Parental	:	= 20000 mg/m³
NOAEL F1 Offspr.	:	= 20000 mg/m³
Method	:	OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year	:	2000
GLP	:	yes
Test substance	:	Volatile fraction of gasoline

Method : Groups of 30 male and 30 female Sprague Dawley rats were exposed 6 hours/day, seven days/week to volatilized test material at target concentrations of 5000, 10 000 and 20 000 mg/m^3.
Singly housed animals were exposed for 10 weeks prior to mating. There was then a 3 week mating period and mating was confirmed by either presence of sperm in a vaginal rinse or by the presence of a vaginal plug.
Exposure of females was continued until gestation day 20. Exposure was then suspended until post partum day 5 to avoid unduly stressing the dams during birth and was then re-commenced and continued until sacrifice of parental females after weaning.
The pups were culled on a random basis to approximately 5/sex/litter. At weaning on postnatal day 28, the F1 pups were selected for the second generation. Among the pups not selected, 3/sex/litter were sacrificed and examined for internal abnormalities. The remainder were examined for external abnormalities, sacrificed and discarded.

The pups selected for F1 were exposed for a 13 week premating period and the for a 3 week mating period as described above.
The males were sacrificed at this time and the females continued to be exposed until gestation day 20. As described above exposures were resumed on post partum day 5 and was continued until weaning, when all remaining animals were sacrificed. Other than during the period from gestation day 20 until post partum day 5, all F1 offspring were exposed from conception to sacrifice.

All animals were examined regularly for viability and clinical observations. Body weights and food intakes were also recorded regularly throughout the study.
All pups were counted and examined externally on a daily basis and weighed at regular intervals until post natal day 21. F1 pups were examined regularly between post natal days 21 to 28 and were weighed on days 28 and 35.
All surviving F1 and F2 pups were examined for developmental landmarks, including pinna detachment, hair growth, incisor eruption, eye opening and the development of the surface righting reflex. Surviving F1 female offspring were monitored for vaginal opening and males were examined for preputial separation.
Reproductive parameters evaluated included: male and female fertility indices, male mating index, female fecundity and gestational indices, mean litter size, mean days of gestation, female estrous cycle length and number of females cycling normally. Live birth index, survival index, survival indices (post partum days 1, 4, 7, 14 and 21), viability index at weaning, mean live and dead offspring on day 0, sex ratio at day 0, offspring in-life observations, offspring body weight and offspring gross postmortem findings were also assessed.

All animals dying or sacrificed in a moribund condition were necropsied.
Culled pups were examined externally but were only necropsied if external evidence warranted it. Randomly selected pups were necropsied and the weight of the following organs was determined: ovaries, liver, adrenals, testes, kidneys, spleen and brain. Additionally a wide range

of tissues were taken for histology.
Similar evaluations were also carried out on all adults surviving to scheduled sacrifice. Tissues taken from the high dose group and controls were evaluated histologically and since there were no untoward findings, tissues from the lower dose groups were not examined.
Samples of sperm from the left distal cauda epididymis were collected from all males at terminal sacrifice for evaluation of sperm parameters. These included assessments of total caudal epididymal sperm numbers, % progressively motile sperm and homogenization resistant spermatid count, % morphologically normal sperm and % sperm with an identified abnormality. An ovarian examination was carried out in the females that included confirmation of growing follicles and corporea lutea and quantification of primordial oocytes. This was done in the high dose and control groups and since there were no abnormal findings other groups were not evaluated.

Result : There were no treatment-related clinical signs, or effects on mortality, body weight or food intake in either parents or pups. Furthermore, there were no treatment-related post mortem findings.

There were no significant differences in absolute organ weights in either males or females from the first parental generation. In the second parental generation, however, there were some statistically significant increases in absolute organ weights, including liver, kidneys and testis in the males and lungs in the females, but none of the differences between controls and the high dose group was statistically significant. In the absence of a clear dose-response relationship the significance of the result was unclear. When expressed as organ/body weight ratios, the only significant difference was seen in male kidney weights in the lowest dose group of the first parental generation and an increase in the highest dose group of the second parental generation. Although this latter may have been treatment related it was not considered to be of clinical importance.

There were no compound-related microscopic changes in any of the reproductive tissues or in the upper or lower respiratory
tract from any of the P1 or P2 rats exposed to 20 000 mg/m3. The only microscopic changes seen were in the kidneys of males of both generations. There was an exposure related increase in the amount and size of hyaline droplets. In three male rats of the high exposure group from both P1 and P2 animals granular casts were observed in the medullary tubules of the kidneys. These kidney changes and the accompanying weight increases are regarded as a sex and species specific effect and of no relevance for man.

In the first generation, there were no differences in mating index, fecundity, pregnancy or length of gestation. Among the offspring there were no differences in litter size, fraction of live births or sex ratio.
Results in the second generation were similar.
There were no differences in survival of offspring through weaning in the first generation and in the second generation early survival was slightly higher among the offspring from the exposed dams.

There were no differences in the weight of the offspring through weaning in either generation.
There were no unusual post mortem observations.

The sperm analysis carried out on both P1 and P2 (F1) males revealed no effects on sperm count, progressive motility or gross appearance.

No effects were found on the estrous cycle length, quantification of primordial oocytes or % females with abnormal cycles in the P1 or P2 generations.

There were no significant differences in incisor eruption, pinna detachment, or surface righting reflex in the F1 or F2 offspring. Hair growth was delayed by just less than one day in males only of the F1 pups and in both sexes of the lowest dose group (approx half day) for the F2 pups. Eye opening was advanced by approximately one-half day for the high dose males of the F2 offspring.

Test substance : The test material was a condensate of gasoline vapor that had been collected from a vapor recovery unit during normal operations. This test material was selected since it was representative of the exposures that normally occur for the general public during self-service refueling. Analytical studies were conducted on the condensate and the results compared with exposure studies that had been carried out during refueling operations. The results confirmed that the vapor recovery condensate was similar in composition to the vapors to which the public are exposed during refueling.

Test atmospheres for the inhalation study were generated by fully volatilizing the condensate and diluting with air to achieve target concentrations of 5000, 10 000 and 20 000 mg/m3. The highest concentration was approximately 50% of the lower explosive limit and several orders of magnitude greater than the concentrations to which the public are exposed..

Chamber analyses of the test atmospheres confirmed the actual concentrations to be: 5076, 10 274 and 20 241 mg/m3.

Analysis of the vapor recovery condensate gave the following results:

Component	Vol %
Non aromatics	
C3	1.0
C4	51.7
C5	37.2
C6	8.3
C7	0.4
C8	0.2
C9	-
C9+	-
Total saturates (vol%)	-
Total olefins (vol%)	-
Aromatics	
Benzene	0.7
Toluene	0.7
C8	-

C9	-
C9+	-
Total aromatics (vol%)	-

NB - denotes no data available.

Reliability : (1) valid without restriction
21.11.2001 (25)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 6 hours each day
Frequency of treatment : Daily
Duration of test : Days 6 through 15 of gestation
Doses : 400 and 1600 ppm
Control group : yes
NOAEL Maternalt. : = 1600 ppm
NOAEL Teratogen : = 1600 ppm
Year : 1978
GLP : no data
Test substance : Unleaded gasoline as described in section 1.1 above
Method : Female rats were mated with sexually mature males of the same strain. The females were examined daily for evidence of a copulatory plug and when this was observed it was designated day 0 of gestation.
The mated female rats were assigned sequentially into three groups of 25 animals for the 0, 400 and 1600 ppm dose groups and were caged individually.
The animals were subjected to whole body exposure to gasoline vapors at the concentrations shown above for 6 hours each day from day 6 through day 15 of gestation.
Mated females were weighed on days 0, 6, 15 and 20 of gestation. Food consumption was recorded daily during the periods 0-6, 6-15 and 15-20 days of gestation. Observations were made daily for clinical signs.
On day 20 of gestation the female rats were anesthetized and their visceral and thoracic organs were examined. The uterus was removed and opened and the number of implantation sites, their placement in the uterine horns, live and dead fetuses and resorption sites recorded.
The fetuses were removed, examined externally for abnormalities and weighed. One third of the fetuses from each litter were fixed in Bouin's and examined later for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses in each litter were stained with Alizarin Red S and examined for skeletal abnormalities.
The uterus and ovaries from the adult females were preserved for possible future examination.
Result : Chamber concentrations were found to be:

Nominal (ppm)	Actual (ppm)
0	0
400	442±42
1600	1573±80

There were no deaths during the study and all animals appeared normal throughout. There were no treatment-related effects on body weight or food consumption.
There were no treatment related effects on any of the reproductive parameters recorded. These data are summarized as follows:

	Historical control	0 ppm	400 ppm	1600 ppm
Pregnancy ratio (pregnant/bred)	-	20/22	22/22	20/21
Live litters	99%	20	22	20
Implantation sites (left /right horn)	46/54%	123/145	149/158	143/152
Resorptions	252	16	22	15
Litters with resorptions	50%	65%	41%	55%
Dead fetuses	1	0	0	0
Litters with dead fetuses	1	0	0	0
Live fetuses/ Implantaion site	92%	95%	93%	95%
Mean live litter size	12.2	13	13	14
Average fetal wt. (g)	3.5	3.8	3.7	3.6

No treatment related effects were observed during the examination for soft tissue changes in the fetuses.
Results of the skeletal examination of the stained fetuses are summarized below:

Dose (ppm)	Fetuses examined	normal	Fetuses with commonly encountered changes only	Fetuses with Unusual skeletal variations
0	177 (20)*	112	60 (18)	5 (5)
400	197** (22)	128	55 (16)	14 (4)
1600	196 (20)	131	47*** (14)	18*** (7)

* Average No. of litters in parenthesis
** Two specimens of one litter lost on processing
*** $p<0.05$

The unusual changes were mainly related to retarded ossification and were not considered as malformations.

Statistical analysis of data on a pup basis revealed a significant difference between the 1600 and 0 ppm groups. However when analyzed on a litter basis no statistically significant differences were found.

Conclusion : Exposure of pregnant rats to vapors of unleaded gasoline at concentrations of 400 or 1600 ppm did not cause effects on pregnant dams. There was no evidence of variation in sex ratio, embryo toxicity, inhibition of fetal growth or development or teratogenic potential.

Reliability : (1) valid without restriction
19.11.2001 (4)

(1) ACGIH (1998)
1998 TLVs and BEIs Threshold Limit Values for Chemical Substances and Physical Agents.

(2) American Petroleum Institute (1977)
Mutagenicity evaluation of unleaded gasoline
Study conducted by Litton Bionetics. Inc.
API HESD Publication No. 28-30173, March 1977

(3) American Petroleum Institute (1977)
Rat bone marrow cytogenetic analysis unleaded gasoline.
Study conducted by Litton Bionetics Inc.
API Med. Res. Publ. 26-60099, November 1977

(4) American Petroleum Institute (1978)
Teratology study in rats unleaded gasoline
Study conducted by Litton Bionetics Inc.
API HESD Res. Publ. 26-60014

(5) American Petroleum Institute (1980)
Acute toxicity tests, API #PS-6 unleaded motor gasoline.
Study conducted by Elars Bioresearch Laboratories Inc.
API Report No. 27-32130.

(6) American Petroleum Institute (1980)
Mutagenicity evaluation of Gasoline, API PS-6 fuel in the mouse dominant lethal assay.
Study conducted by Litton Bionetics Inc.
API Publication No. 28-31344. April 1980

(7) CONCAWE (1992)
Ecotoxicological Testing Of Petroleum Products : Test Methodology.
Report 92/56,
CONCAWE, Brussels.

(8) CONCAWE (1995)
Algal, Growth Inhibition Test: study no. 104867, test substance MRD-95-048.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995

(9) CONCAWE (1995)
Algal, Growth Inhibition Test: study no. 104967, test substance MRD-95-049.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(10) CONCAWE (1995)
Daphnia - acute toxicity test: study no. 104842, test substance MRD-95-048.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(11) CONCAWE (1995)
Daphnia - acute toxicity test: study no. 104942A, test substance MRD-95-049.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(12) CONCAWE (1995)
Fish - acute toxicity test: study no. 104858, test substance MRD-95-048.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(13) CONCAWE (1995)
Physico-chemical characterization of gasoline samples, study no. 104990C. Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(14) CONCAWE (1996)
Environmental risk assessment of petroleum substances: the hydrocarbon block method.
Report 96/52,
CONCAWE, Brussels.

(15) ECETOC (1998)
QSARS in the Assessment of the Environmental Fate and Effects of Chemicals. Technical Report No. 74

(16) ECETOC, (1996)
Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances. Monograph 26.

(17) Harris, J.C. (1982)
Rate of Hydrolysis. In Handbook of Chemical Property Estimation Methods. p. 7-6. W. J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY, USA.

(18) IRDC (1984)
Motor fuel chronic inhalation study. Unleaded gasoline. Amendment to the final report. Study sponsored by API
IRDC 418-003.

(19) Kitchen, D (1984)
Neoplastic renal effects of unleaded gasoline in Fischer 344 rats.
In: Advances in Modern Environmental Toxicology Volume VII: Renal effects of petroleum hydrocarbons, pages 65-71
Princeton NJ

(20) Kuna,R. A. and Ulrich, C. E. (1984)
Subchronic inhalation toxicity of two motor fuels.
J. American College of Toxicology. Vol 3. No. 4. 217-229.

(21) MacFarland, H. N. (1984)
Xenobiotic induced kidney lesions: Hydrocarbons. The 90-day and 2-year gasoline studies.
In: Advances in Modern Environmental Toxicology Volume VII: Renal effects of petroleum hydrocarbons, pages 51-57
Princeton NJ

(22) MacFarland, H. N., Ulrich, C. E., Holdsworth, C. E., Kitchen, D. N., Halliwell, W. H. and Blum, S. C. (1984)
A chronic Inhalation Study with unleaded gasoline vapor.
J. Am. College of Toxicol. Vol. 3, No. 4, pp 231-248

(23) Mackay, D, A. DiGuardo, S. Paterson, & C. Cowan (1997) EQC Model, ver. 1.01, 1997, available from the Environmental Modelling Centre, Trent University, Canada.

(24) McFarland, H. N., Ulrich, C. E., Holdsworth, C. E., Kitchen, D. N., Halliwell, W. H. and Blum, S. C. (1984) A chronic Inhalation Study with unleaded gasoline vapor. J. Am. College of Toxicol. Vol. 3, No. 4, pp 231-248

(25) McKee, R. H., Trimmer, G. W., Whitman, F. T., Nessel, C. S., Mackerer, C. R., Hagemann, R., Priston, R. A. J., Riley, A. J., Simpson, B. J. and Urbanus, J. H. (2000) Assessment in rats of the reproductive toxicity of gasoline from a gasoline vapor recovery unit. Reproductive Toxicology Vol 14, No. 4, pp 337-353

(26) Meylan, M, SRC 1994-1999. LOGKOWWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(27) Meylan, M, SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(28) Solano-Serena, F., R. Marchal, D. Blanchet, and J.-P. Vandecasteele. 1998. Intrinsic Capacities of Soil Microflorae for Gasoline Degradation. Biodegradation 9:319-326.

(29) Solano-Serena, F., R. Marchal, M. Ropars, J.-M. Lebeault, and J.-P. Vandecasteele. 1999. Biodegradation of Gasoline: Kinetics, Mass Balance and Fate of Individual Hydrocarbons. J. Appl. Microbiol. 96:1006-1016.

(30) Springborn Laboratories, Inc.(1993). CWE5 (Blended Gasoline) Toxicity to Freshwater Alga, Selenastrum capricornutumSLI Report # 93-5-4783.Springborn Laboratories, Inc Environmental Sciences division, 790 Main Street, Wareham, Massachusetts, USA

(31) Study conducted by Exxon Biomedical Sciences Inc. Fish - acute toxicity test: study no. 104958, test substance MRD-95-049. CONCAWE, Brussels, 1995

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # NAPHTHENIC NAPHTHAS

Summary prepared by: American Petroleum Institute

Creation date: **22 FEBRUARY 2001**

Printing date: **15 NOVEMBER 2001**

Date of last Update: **10 DECEMBER 2001**

Number of Pages: **28**

NB. Reliabiliy of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : petroleum product
Physical status : liquid
Remark : The naphtha streams that are rich in naphthenes are obtained from the atmospheric distillation of crude oil. The streams contain saturated and aromatic hydrocarbons, mainly in the range C_4 to C_{12} and boil in the range of approximately minus 20 to 230 °C

Sweetened Naphtha (CAS No. 64741-87-3) is typical of the streams in this category.

An API sample of Sweetened naphtha that has been used for some of the toxicology studies for this group has been characterized as follows:

SAMPLE API 81-08

Gravity (°API)	76.6
Sulfur (wt %)	0.1
Nitrogen (ppm)	<1
RVP (psia)	01.2
IBP (°F)	98
FBP (°F)	262
Paraffins (% by MS)	76.5
Olefins (% by MS)	1.0
Naphthenes (% by MS)	16.5
Aromatics (% by MS)	4.0
Saturates (% by MS)	-

12.11.2001

2.1 MELTING POINT

: Not relevant

2.2 BOILING POINT

Value : Approximately 49 - 177 ° C at 1013 hPa
Decomposition : no
Remark : The samples, which were used by the API in its toxicity assessments for this Group were prepared by the fractionation of two types of crude oil, using a pilot plant still and separating cuts in a distillation range of 120 to 350°F (49 to 177°C).

These figures represent a typical boiling range for light straight-run naphtha, CAS No. 64741-46-4.

The standard oil industry method for determination of boiling range is ASTM D86.

27.10.2001 (10) (18)

2.5 PARTITION COEFFICIENT

Log pow : 2.13 - 4 at 25° C
Method Calculated by LOGKOWWIN ver. 1.65
Year : 2000
GLP : no
Test substance : Light Straight Run Naphtha (LSRN)-Low Naphthenic, CONCAWE sample CWE3, CAS No. 64741-46-4
Remark : Log P values represent the spread of calculated and/or measured values for C_5 to C_7 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability : (2) valid with restrictions
12.11.2001 (13) (20)

Log pow : 2.13 - 4.76 at 25° C
Method Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : no
Test substance : Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, CAS No. 64741-46-4
Remark : Log P values represent the spread of calculated and/or measured values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific moderate naphthenic (19.7%) LSRN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.
Reliability : (2) valid with restrictions
11.11.2001 (20)

Log pow	:	2.73 - 4.85 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65
Year	:	2000
GLP	:	No
Test substance	:	Light Straight Run Naphtha (LSRN)-Hi Naphthenic, CONCAWE sample W94/809, CAS No. 64741-46-4
Remark	:	Log P values represent the spread of calculated and/or measured values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability	:	(2) valid with restrictions

27.10.2001 (13) (20)

2.6.1 WATER SOLUBILITY

Method	:	Preparation of Water Soluble Fractions
Year	:	1995
GLP	:	yes
Test substance	:	Light Straight Run Naphtha (LSRN)-Hi Naphthenic, CONCAWE sample W94/809, CAS No. 64741-46-4
Method	:	Water Accomodated Fractions (WAFs) of LSRN were prepared at 100 mg/L loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of TEX (toluene, ethyl benzene, and xylenes) components indicated freshwater solubility of 5.7-7.9 ppm (as TEX). Measured test concentrations of the LSRN were based on the total combined concentrations of TEXN which represent approximately 13% composition of the test substance. Concentrations for these components reached equilibrium by 19 hours.
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) valid with restrictions

27.10.2001 (11) (12) (14) (15) (16)

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	yes
Test substance	:	Light Straight Run Naphtha (LSRN)-Low Naphthenic, CONCAWE sample CWE39, CAS No. 64741-46-4
Method	:	Water Accomodated Fractions (WAFs) of LSRN were prepared at 1000 mg/l loading in freshwater and equilibrated for 24 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of BTEX components indicated freshwater solubility at 24 hours of 4.9 ppm as benzene.

Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for gasoline components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) valid with restrictions
27.10.2001		(11) (14) (15) (16) (22)
Method	:	Preparation of water soluble fraction
Year	:	1995
GLP	:	Yes
Test substance	:	Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, CAS No. 64741-46-4
Method	:	Water Accomodated Fractions (WAFs) of LSRN were prepared at 50 mg/L loading in freshwater and saltwater and equilibrated for 48 hours in tightly closed systems with minimal headspace
Result	:	Gas chromatographic analysis of LSRN components 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene in WAFs indicated freshwater solubility of 1.62-1.81 ppm and 1.50 mg/l in saltwater. Concentrations for these components reached equilibrium by 24 hours.
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) valid with restrictions
11.11.2001		(4) (11) (14) (15) (16)

3.1.1 PHOTODEGRADATION

Type	:	Calculated
Light source	:	Sun light
Indirect photolysis		
Sensitizer	:	OH
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	No
Test substance	:	Light Straight Run Naphtha (LSRN)-Low Naphthenic, CONCAWE sample CWE39, CAS No. 64741-46-4
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 1.262 days (cyclohexane) to 15.985 days (isopentane).
Result	:	Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate Constant: 0.6991 E-12 (isopentane) to 8.4783 E-12 (cyclohexane) cm^3/molecule-sec Half-life 1.262 to 15.985 days
Reliability	:	(2) valid with restrictions
27.10.2001		(13) (21)

Type	:	Calculation
Light source	:	Sun light
Indirect photolysis		
Sensitizer	:	OH
Deg. Product	:	
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	No
Test substance	:	Light Straight Run Naphtha (LSRN)-Hi Naphthenic, CONCAWE sample W94/809, CAS No. 64741-46-4
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 0.789 days (m-xylene) to 5.486 days (benzene).
Result	:	Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate Constant: 1.9498 E-12 (benzene) to 13.5606 E-12 (m-xylene) cm^3/molecule-sec Half-life: 0.789 to 5.486 days
Reliability	:	(2) valid with restrictions
27.10.2001		(13) (21)

Type	:	Calculation
Light source	:	Sun light
Rel. intensity	:	1 based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Method	:	Calculated Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	No
Test substance	:	Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, CAS No. 64741-46-4
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific moderate naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 0.789 days (m-xylene) to 15.985 days (isopentane).
Result	:	Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate Constant: 0.6691E-12 (isopentane) to 13.5606E-12 (m-xylene) cm^3/molecule-sec Half-life: 0.789 to 15.985 days
Reliability	:	(2) valid with restrictions

11.11.2001 (21)

3.1.2 STABILITY IN WATER

Test substance	:	Light Straight Run Naphtha (LSRN)-Hi Naphthenic, CONCAWE sample W94/809, CAS No. 64741-46-4
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) valid without restriction

27.10.2001 (17)

Test substance	:	Light Straight Run Naphtha (LSRN)-Low Naphthenic, CONCAWE sample CWE39, CAS No. 64741-46-4
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) valid without restriction

27.10.2001 (17)

Test substance	:	Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, CAS No. 64741-46-4
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) valid without restriction

11.11.2001 (17)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment, sediment
Year : 2000
Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample.

The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

Medium	% distribution
Air:	97 to 99.97
Soil:	0.03 to 1.2
Water:	0.008 to 2.7
Sediment	0.00 to 0.02
Suspended sediment	0.00

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.
Reliability : (2) valid with restrictions
24.09.2001 (13) (19)

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment, sediment
Method : Calculated
Year : 2000
Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample.

The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

Medium	% distribution
Air:	98.89 to 99.98
Soil:	0.01 to 0.11
Water:	0.01 to 1.00
Sediment	<0.001
Suspended sediment	<0.001

Reliability : (2) valid with restrictions
27.10.2001 (13) (19)

Type	:	Calculated according to Mackay Level 1
Media	:	Soil, air, water, suspended sediment, sediment
Method	:	Calculated
Year	:	2000
Remark	:	Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific moderate naphthenic (19.7%) LSRN sample. The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.
Result	:	

Medium	% distribution
Air:	97 to 99.99
Soil:	0.00 to 1.2
Water:	0.013 to 2.7
Sediment	0.00 to 0.03
Suspended Sediment	0.00

Test substance	:	Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, No. 64741-46-4
Conclusion	:	The constituents of this complex petroleum mixture are expected to partition primarily to air.
Reliability	:	(2) valid with restrictions

11.11.2001 (19)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static with daily renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Year	:	1996
GLP	:	Yes
Test substance	:	Light Straight Run Naphtha
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002. LL_{50} and LC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 6, 20 and 21 in 0, 3.1, 6.3, 13, 25 and 50 mg/l treatments. Abnormal behavior (surfacing, erratic swimming, quiescence) was observed at 96 hrs for 6 organisms in the 13 mg/l treatment. 96-hr LL_{50} = 15 mg/l, 6.3-25 mg/l w/ 95% C.I. (as nominal loading rate) 96-hr LC_{50} = 0.689 mg/l, 0.289-0.962 mg/l w/ 95% C.I. (measured concentrations) 96-hr NOEL = 6.3 mg/l (nominal); 96-hr NOEC =0.287 mg/l (measured) based on lack of mortality and abnormal effects for these treatments. A low boiling point naphtha sample w/ CAS no. 8030-30-6 (different from the sample used in toxicity testing, but similar in composition) was used to validate the analytical method being developed to identify water-soluble hydrocarbons in aqueous 24-hour equilibrated samples. This does not appear to have affected the results of the study.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by blending naturally hard well water with water that had been demineralized by reverse osmosis. Nominal loading rates of 0, 3.1, 6.3, 13, 25 and 50 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a Teflon lined neoprene stopper housing two Teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, Para film was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes. Fish were hatched and raised from ABC Laboratories' in-house culture, and were acclimated prior to experimentation for a minimum of 14 days on

a 16/8hr light/dark cycle. Test vessels were 3.8 liter glass containers with Teflon lined caps. Fish were acclimated to the test water and temperature approximately 72 hr before the test, and were not fed during this 72 hr period. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control, with the exception of the 50 mg/l treatment, where 11 organisms instead of 10 were placed in one replicate. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving approximately one liter of solution to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Water temperature was 21-22 °C. Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 7.3 and 8.8, pH values between 8.1 and 8.3. Hardness values ranged from 134 to 144 mg/l; alkalinity values ranged from 144 to 154 mg/l and conductivity values ranged from 300 to 340 microsiemens.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

12.11.2001 (3)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Method	:	Procedure patterned after:1991 ASTM method E729-88a and 1985 USEPA TSCA Test Guidelines: Daphnid Acute Toxicity Test. Fed. Reg., vol. 50 (No. 188) Sept 27, 1985, 797.1300.
Year	:	1996
GLP	:	yes
Test substance	:	Light Straight Run Naphtha
Method	:	Statistical Method: (FT - ME) EL_{50} and EC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Immobility (no. of organisms) at 48 hrs: 1, 3, 0, 0, 19 and 20 for 0, 3.0, 6.0, 12, 24 and 48 mg/l treatments. At the 3 and 12mg/l nominal treatments, 1 and 20 organisms were observed at the bottom of the test chambers, respectively. 48-hr EL_{50} = 18 mg/l based upon nominal loading rate (95% C.I. 12 to 24 mg/l) 48 hr EC_{50} was 0.65 mg/l (95% C.I. 0.47 to 0.83 mg/L); based on total measured concentrations. 48-hr NOEL = 6.0 mg/l based upon nominal loading rate. 48 hr NOEC was 0.24 ppm based on total measured concentrations.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were a blend of aged well water and reverse osmosis well water. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 2.4L of water for 24 hr in aluminum foil covered 2.5 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles

were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately one hour, then drained from the port and used for testing. Samples were also analyzed by purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes.

Range finding toxicity studies were conducted at 0.5, 1.0, 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 3.0, 6.0, 12, 24 and 48 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.

Test vessels were Teflon cap-sealed 8 oz. glass jars with10 daphnids per jar and were completely filled to overflowing with approximately 273 ml test solution.

During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.5; pH ranged from 8.3 to 8.4; temperature was 20 to 21 °C; hardness (mg/l) ranged from 132 - 140; alkalinity (mg/l) was 142-150 and conductivity (umhos) values were 280 - 300.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from 11 day culture maintained in-house since October 1996.

Reliability : (2) valid with restrictions: Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitiated to determine total measured concentrations.

27.10.2001 (1)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year	:	1997
GLP	:	yes
Test substance	:	Light Straight Run Naphtha
Method	:	Statistical Method: EL_{50} and EC_{50} calculated using nonlinear logistics sigmoid model (SAS). All NOEL/NOEC values based on visual review and Dunnett's test for significance.
Result	:	Percent inhibition on growth determined by cell density (cells/ml): 96 hour EL_{10}=2.7 mg/l (1.9-3.5 mg/l CI @95%) 96 hour EL_{50}=6.4mg/l (5.7-7.1 mg/l CI @95%) 96 hour EL_{90}=15 mg/l (12-18 mg/l CI @95%) 96 hour NOEL=1.9 mg/l 96 hour EC_{10}=0.1 mg/l (0.061-0.15 mg/l CI @95%) 96 hour EC_{50}= 0.26 mg/l (0.22-0.30 mg/l CI @95%) 96 hour EC_{90}=0.66 mg/l (0.50-0.83 mg/l CI @95%) 96 hour NOEC=0.0326 mg/l Subcultures of the 31 mg/l treatment cultures were placed in fresh media (no test substance) after acute testing for ten days and indicated that

growth inhibition was algistatic in this treatment. Conduct of the range-finder and definitive tests were accepatable (no repeats). No excursions from the protocol were noted which would have affected the integrity of the study.

Nominal (mg/l) Conc (meas mg/l).	96hr cell density (cells/ml)
Control (<LOQ)	43.58 $x10^4$
1.9 (0.0322)	42.332 $x10^4$
4.0 (0.130)	29.25 $x10^4$
7.8 (0.329)	18.42 $x10^4$
16 (0.704)	1.74 $x10^4$
31 (1.29)	0.04 $x10^4$

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 9.4-9.6l of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 µg/l EDTA chelator, pH adjusted to 7.5 + 0.1 with 0.1 NHCl and sterilized by 0.45 micron filtration) in 9.5 liter aspirator bottles. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a Teflon lined neoprene stopper housing two Teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, Para film was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. The contents were stirred with Teflon coated stir bars in the mixing vessels which were placed on magnetic stir plates at room temperature. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (148 ml) with treatment solution and inoculated with 3 day old algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media. Original algal cultures (stock UTEX-1648) obtained from Dept of Botany, Culture Collection of Algae, University of Texas at Austin, 1996. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 1.9, 4.0, 7.8, 16 and 31 mg/l The initial algal concentration was 1.0 x 103 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination 400 +50 ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0,24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes.

Test temperature was 24-26 °C. Test solution pH ranged from 8.0 to 8.5.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

27.10.2001 (2)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	undiluted
Value	:	> 5000 mg/kg bw
Year	:	1982
GLP	:	yes
Test substance	:	API 81-08
Method	:	The test material was administered undiluted, as a single oral dose to groups of 5 male 5 female rats at a single dose level of 5 g/Kg. The dose volume was 7.35 ml/Kg based on an average bulk density of 0.68 g/ml. Food had been withheld from the rats overnight prior to dosing, but they had free access to water. Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days. The rats were weighed the day before dosing and then at 7 and 14 days after dosing. At study termination, all animals were killed with carbon dioxide and subjected to a gross necropsy and abnormalities were recorded.
Result	:	No animals died during the study. Clinical signs of intoxication included diarrhea and mucoid diarrhea. Although there was a reduction in body weight following fasting, body weights were increasing by seven and 14 days post dosing. At necropsy, no visible lesions were observed in 4 of 5 males and 2 of 5 females. In the right kidney of one male, the renal pelvis was mildly dilated and a cervical lymph node was enlarged. In the females dilation of the pelvis of the kidney was observed in one animal, a cervical lymph node was enlarged in another animal and in a third animal mild hydrometra of the uterus was observed. The Oral LD50 was greater than 5 g/Kg.
Reliability	:	(1) valid without restriction

27.10.2001 (5)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	air
Exposure time	:	4 hour(s)
Value	:	> 5.2 mg/l
Year	:	1986
GLP	:	yes
Test substance	:	API 81-08
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 81-08 at a nominal concentration of 5mg/l for 4 hours. After the 4-hour exposure, the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14, all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.
Result	:	The actual chamber concentrations were found to be 5.2 mg/l. No deaths occurred during the study. There were no unusual pharmacotoxic signs or behavior observed in the control animals. There was however, a slight incidence of nasal discharge (2/5 males and 1/5 females) during the exposure period but none during the following 14-day observation period. The body weight gains for the males exposed to API 81-08 was considered normal but the female body weight gains were marginally less than that of the controls on day 14 post exposure (8.2% compared to 13.8% increase over pre-exposure body weight). No significant macro or microscopic changes were observed that were considered treatment related.
Reliability	:	(1) valid without restriction

27.10.2001 (7)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	8
Vehicle	:	undiluted
Value	:	> 2000 mg/kg bw
Year	:	1982
GLP	:	yes
Test substance	:	API 81-08
Method	:	The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to a dose of 2 g/Kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. Collars were used to restrain the animals during the application period. The animals were observed for a total of 14 days post-dosing. Body weights were recorded just prior to dosing and again seven and 14 days after dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Result	:	No animals died during the study and no clinical signs of intoxication were observed. Normal growth was observed throughout the study. At necropsy, the only visible lesions seen were on the skin of two animals in which the test site was reddened in one together with crusted appearance and mild crusting was observed in the other rabbit.
Reliability	:	(1) valid without restriction

27.10.2001 (5)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
PDII	:	1.2
Result	:	slightly irritating
Method	:	Draize Test
Year	:	1982
GLP	:	yes
Test substance	:	API 81-08
Method	:	0.5 ml of undiluted test material was applied to two areas on each rabbit. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing. After 24 hours, the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree

of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours again at 96 hours, 7 and 14 days. Results of the 24 and 72-hour readings were used to determine the Primary Irritation Index.
Body weights were recorded just prior to application of the test material and weekly thereafter throughout the study.
At study termination, all surviving animals were euthanized with an overexposure of carbon dioxide, subjected to a gross necropsy and abnormalities were recorded.

Result : The scores for erythema and edema at each of the observation times were as follows:

	Erythema		Edema	
	Intact	Abraded	Intact	Abraded
24 h	0.7	0.7	1.0	0.7
72 h	1.0	0.7	0	0
96 h	0.5	0.2	0	0
7 days	0.3	0.3	0	0
14 days	0	0	0	0

The Primary dermal Irritation index was 1.2

Growth rates were normal throughout the study and there were no visible lesions at necropsy.

Reliability : (1) valid without restriction
27.10.2001 (5)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : 0.1 ml
Comment : rinsed after 30 sec exposure in 3 rabbits; 6 rabbits unwashed
Number of animals : 9
Result : not irritating
Method : Draize Test
Year : 1982
GLP : yes
Test substance : API 81-08
Method : 0.1 ml of undiluted test material was placed in the everted lower eyelid of one eye of each of 9 rabbits, the other eye was untreated and served as control.
After 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed.
Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. At the 72 hour and seven day readings, sodium fluorescein was used to aid in revealing possible corneal injury.
Body weights were recorded just prior to treatment and one week afterwards. At termination of the study, the rabbits were euthanized by an overexposure of carbon dioxide and were subjected to a gross necropsy. Any abnormalities found were recorded.

Result : One hour after application of the test material the average score for irritation was 2.0 and 0.7 for unwashed and washed eyes respectively and the 24 hour readings were 0.3 and 0 respectively. All other scores throughout the study were 0.
Growth was normal throughout the study and there were no visible lesions at necropsy.

Reliability : (1) valid without restriction
27.10.2001 (5)

5.3 SENSITIZATION

: No data

5.4 REPEATED DOSE TOXICITY

: No data

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Mouse lymphoma assay
System of testing	:	Forward mutation assay using cell line L5178Y TK+/-
Concentration	:	12.5 - 300 µl/ml
Cycotoxic conc.	:	0.05µl/ml without activation; 0.5µl/ml with activation
Metabolic activation	:	with and without
Result	:	negative
Year	:	1985
GLP	:	yes
Test substance	:	API 81-08
Method	:	Based on a preliminary test, ethanol was selected as solvent for this assay. Concentrations of 0.061 to 1000 µl/ml appeared soluble in the assay medium and no change in color was noted. Two positive control substances were used viz Ethyl methane sulphonate (EMS) at a concentration of 0.5 µl/ml in the assay without activation and Dimethylnitrosamine (DMN) at a concentrations of 0.3 µl/ml. A cytotoxicity study was carried out prior to the mutagenicity assay. The results were difficult to interpret and as a consequence a second study was carried out and the results from this were used to determine the concentrations to be used in the subsequent lymphoma assay. It was established that complete toxicity occurred at 0.05 µl/ml for the non-activated cultures and at 0.5 µl/ml for S-9 activated cultures. For the mutation assay the lymphoma cells were exposed for 4 hours to test material at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; TFT was used as the restrictive agent. Eight non-activated and nine activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 0.005, 0.01, 0.015, 0..02, 0.025, 0.03, 0.035 or 0.04 µl/ml and resulted in a range of growth of 6 to 97%. The activated cultures that were cloned were treated with 0.0002, 0.0009, 0.0028, 0.008, 0.02, 0.045, 0.09, 0.7 or 0.75 µl/ml and produced a range of growth from 24 to 109%. Plates were prepared from TFT and from the VC cultures and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was

then determined.

The following criteria were used in judging the significance of the activity of the test article.

Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Result : The data from each of the 3 trials that were considered valid are tabulated below.

Conc. (µl.ml)	Relative growth (%)	Mutant frequency (10-6 units)
TRIAL 1 (No activation)		
15.6	118.6	18.1
31.3	64.4	27
62.5	97.8	15.7
125	78.2	24.2
250	20.1	48.8
Solvent control 1	100	13.9
Solvent control 2	100	20.3
Untreated control	191.7	21.5
EMS 0.5 µl/ml	17.4	258.2
TRIAL 1 (with S-9 activation)		
15.6	78.1	59.1
31.3	53.8	49.3
62.5	63.3	49
125	46.5	79.7
250	46.3	41.6
Solvent control 1	100	34
Solvent control 2	100	24
Untreated control	100.7	30.5
DMN 0.3 µl/ml	5	327.5
TRIAL 4 (No activation)		
12.5	47.8	19.5
25	49.7	19.2
50	37.7	13.5
100	113.3	8.5
200	86.2	9.3
300	19.8	36.4
Solvent control 1	100	18.3
Solvent control 2	100	18.5
Untreated control	163.9	16.2
EMS 0.5 µl/ml	13.5	700
TRIAL 4 (with S-9 activation)		
12.5	81.6	52.3
25	60.2	85.7
50	57.3	59.1
100	44.7	63.8
200	71.8	21

300	3.1	19.3
Solvent control 1	100	23.2
Solvent control 2	100	22.9
Untreated control	78.2	22.2
DMN 0.3 µl/ml	8.8	469.4

TRIAL 5 (with S-9 activation)

150	76.9	13.6
150	28.4	25.2
200	42.5	24
200	41.9	15.3
250	59.6	24.2
250	15.6	31.1
300	4.9	30.2
300	7.3	32
Solvent control 1	100	27.1
Solvent control 2	100	19.2
Solvent control 3	100	22.4
Solvent control 4	100	24.5
Untreated control 1	63.8	31
Untreated control 2	49.9	29.2
DMN 0.3 µl/ml	16.6	352.9
DMN 0.3 µl/ml	2.2	333.3

The authors judged the data using the following criteria.

TRIAL 1
Non activation conditions.
The percent relative growths of the assayed treatments ranged from 118.6% to 20.1% which demonstrated non-detectable to moderate toxicities.
The minimum criterion for mutagenesis in this assay was a mutant frequency that exceeding 37.8x10-6. The highest, most toxic treatment (250 µl/ml) induced a mutant frequency that exceeded the minimum criterion, but the increase in the mutant frequency was not accompanied by an increase in the total mutant clones. In order to determine if the increase was repeatable, another non-activation assay was performed.

Activated assay
Test material was assayed at concentrations ranging from 15.6 to 250 µl.ml. The minimum criterion for mutagenesis in his assay was a mutant frequency exceeding 54.2x10-6.
Two treatments induced mutant frequencies that exceeded the minimum criterion, but the increases were sporadic and unrelated to dose or toxicity. Another assay was therefore performed.

TRIAL 4
Non activated assay.
The test material was assayed at concentrations ranging from 12.5 to 300 µl/ml. In order for a treatment to be considered mutagenic in this assay, a mutant frequency of 36.5x10-6 was required. None of the assayed treatments induced mutant frequencies that exceeded the minimum criterion. The observed toxicities ranged from non toxic to moderate toxicity. Although it is preferable to consider results from treatments that induce high toxicity, it was not possible in this assay because of a sharp toxicity curve. The test material was therefore considered non mutagenic without activation in this assay at treatments that approached lethality.

Activated assay.
Concentrations ranging from 12.5 to 300 µl/ml were used in this assay and low to very high toxicity was induced.
Sporadic increases in the mutant frequency were induced. The minimum criterion for mutagenesis in this assay was a mutant frequency exceeding 44.2x10-6 and three treatments did exceed the minimum criterion (25, 50 & 100 nl/ml). However, the highest concentrations assayed were non-mutagenic. A further assay was therefore performed.

TRIAL 5
Activated assay
The test material was assayed in duplicate at concentrations ranging from 150 to 300 nl/ml.
A wide range of toxicities were induced.
The sporadic increases in mutant frequency observed in Trials 1 and 4 were not repeatable. None of the treatments induced mutant frequencies that exceeded the minimum criterion of 48.4x10-6.
The test material was therefore considered non-mutagenic with activation in this assay.

Reliability : (2) valid with restrictions (multiple assays needed to get usable studies)
12.11.2001 (6)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hours/day, for 5 days
Doses	:	0, 65, 300 & 2050 ppm, nominal concentrations
Result	:	negative
Year	:	1986
GLP	:	yes
Test substance	:	API 81-08
Method	:	Groups of 10 male and 10 female Sprague Dawley rats were exposed (whole body) to nominal concentrations of 65, 300 and 2050 ppm of test material. Exposures were exposed to vapor of the test material 6 hours each day for 5 consecutive days. A positive control group of 10 rats of each sex was given a single dose (0.8 mg/kg) of TEM intraperitoneally 24 hours before sacrifice. A negative control group of 10 rats of each sex was exposed to air only. For the treated and negative control groups bone marrow was harvested 6 hours after the final exposure. For the positive control group the bone marrow was harvested 24 hours after administration of the TEM. Three hours prior to sacrifice by carbon monoxide the rats were given a single intraperitoneal dose of colchicine (4 mg/kg). Immediately after sacrifice, bone marrow was obtained from the tibiae of the animals. The marrow was washed and the cells were fixed before being spread on slides for examination. Routinely 50 spreads were prepared for each animal. The location of cells bearing aberrations was identified. A

mitotic index based on at least 500 cells counted was also recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.
Slides were scored for chromosomal aberrations.

The authors give the following as the criteria for a positive response and data interpretation.
Gaps were not counted as significant aberrations.
Indicators of genetic damage were considered to be: Open breaks, configurations resulting from the repair of breaks. The latter included translocations, multiradials, rings,
multicentrics etc. Reunion figures such as these were weighted slightly higher than breaks since they usually resulted from more than one break.
The number of cells with aberrations per animal was also considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of mutagenic potential.
Often it is not possible to locate 50 suitable metaphase spreads for each animal, even after preparing additional spreads. Possible causes for this appear to be related to cytotoxic effects, which alter the duration of the cell cycle, kill the cell or cause clumping of the chromosomes.
Additional information can be gained from the mitotic index, which also appears to reflect cytotoxic effects.
The type of aberration, its frequency and its correlation to dose in a given time period was considered in evaluating a test article as being mutagenically positive or negative.
Statistical analysis employed a Kruskal-Wallis test of aberrations per cell on a per animal basis.

Result **:** The mean exposure chamber concentrations were found to be: 0, 69±18, 293±42 and 2012±16 ppm.

No signs of toxicity were observed in the rats during the exposure phase of the study.
The results of the cytogenetic evaluation are summarized in the following table. NB. Mean values without standard errors are given in the table, although these data are available in the report.

	Exposure concentration (ppm)			Control	
	69	293	2012	Positive	Negative
Total No. of cells					
Male	470	500	410	400	500
Female	500	500	500	474	500
M+F	970	1000	910	874	1000
Frequency of structural aberrations					
Male	.009	.006	.029	>.708	.016
Female	0	>.014	.030	>.970	.008
M+F	.005	>0.01	.029	>.853	.012
Frequency of numerical aberrations					
Male	.012	0	.013	.023	.01
Female	.012	.016	.006	.015	.008
M+F	.01	.008	.01	.019	.009
% Cells with structural aberrations/animal					
1 or more					
Male	.9	.4	2.2	20	.6

Female	0	1.4	2.6	19	.8
M+F	.5	.9	2.4	19.5	.7
2 or more					
Male	0	.2	.4	11.3	.4
Female	0	.2	.4	14.4	0
M+F	0	.2	.4	12.9	.2
%MI					
Male	6.5	6.6	3.8	1	5.7
Female	4.1	4.8	4.5	1	4.2
M+F	5.3	5.7	4.2	12.9	.2

On the basis of the above data, the authors concluded that there was no evidence of a clastogenic effect of the test material and that there was no significant increase in chromosomal aberration in the dosed animals when compared to the negative controls.

Reliability : (1) valid without restriction
12.11.2001 (8)

5.7 CARCINOGENITY

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : Lifetime
Frequency of treatment : Twice weekly.
Post. obs. period : None
Doses : 50 µl/application
Control group : Untreated, solvent and positive controls
Year : 1989
GLP : Yes
Test substance : API 81-08
Method : The study summarized here was designed to evaluate the carcinogenicity of 12 different petroleum refinery streams. Only the information relating to the control groups and the group exposed to API 81-08 is included in this summary.

50 µl of undiluted test material was applied twice weekly to the shorn dorsal skin to a group of 47 male mice for 139 weeks. An untreated group of 50 male mice served as untreated controls. A further 50 male mice used as solvent controls received 50 µl of toluene twice weekly for 2 years and BaP at concentrations of 0.01% and 0.05% in toluene was applied twice weekly to a further two groups of 50 male mice.
Body weights of the mice were recorded prior to study initiation, weekly for the first 13 weeks of the study and every 4 weeks until termination at 139 weeks.
Observations were made daily for morbidity, mortality and any clinical signs of toxicity. All tumors that developed were recorded and their progression noted.
A gross necropsy was performed on all animals dying during the study or killed at termination. Special attention was paid to any dermal and subcutaneous masses.

Liver, kidneys, lungs and gonads were weighed for each animal at necropsy and group mean organ weight and organ/body weight ratios were calculated.
The test skin site (including dermal and subcutaneous tumors) and control skin site were examined histopathologically as were any suspected dermal and systemic neoplasms.

Result : The body weights of the mice treated with 81-08 did not differ from those of controls throughout the study. No clinical signs of systemic toxicity were observed in animals treated with 81-08. Observations of preputial gland swelling and penile prolapse increased in all groups with age. Penile prolapse occurred in virtually all mice by 2 years.

Virtually no dermal lesions were observed in the untreated control group. However, mice treated with toluene had an average of 100% incidence of mild or moderate desquamation and an average of 10 to 20% incidence of mild irritation and scabbing. The incidence of scabbing increased u to 40% in older mice.

Dermal lesions in mice treated with 0.01% BaP were similar to the toluene controls. Although they initially had less irritation than the animals treated with toluene, the incidence of mild irritation increased to approximately 50% after 2 years.
To begin with, the mice treated with 0.05% BaP had similar lesions to the toluene controls. However, the incidence of irritation increased to 50 to 100% from weeks 60 to 78.

Dermal lesions in mice treated with 81-08 were very similar to the toluene controls, but with slightly less irritation and scabbing.
Survival of the mice treated with 81-08 was better than that for any of the control groups as shown in the following table

Group	Survival % at month				
	6	12	18	24	30
Untreated	90	90	86	62	18
Toluene	96	94	76	52	10
BaP 0.01%	100	98	84	38	0
BaP 0.05%	100	86	2	0	0
81-08	98	98	89	56	19

A variety of non-neoplastic lesions other than those at the treated skin site were observed at histopathological examination but these occurred in all groups and were not considered to be treatment related.
Lesions at the treated skin site are summarized as follows:

Lesion			Group		
	Solvent control	Toluene control	BaP 0.01%	BaP 0.05%	81-08
Dermal inflammation					
% affected	0	36	20	24	6
Severity		1.7	1.6	1.6	1.3
Hyperkeratosis					
% affected	0	96	90	92	94
Severity		2	2	2	2

Acanthosis					
% affected	0	76	80	78	87
Severity		1.8	1.7	1.7	1.9
Epidermal crusting					
% affected	2	84	82	86	75
Severity	1	1.8	1.7	2.1	1.9
Dermal pigmentation					
% affected	0	78	12	2	87
Severity		1.6	1	1	1.9
Dermal fibrosis					
% affected	0	68	54	36	96
Severity		1.6	1.4	1.4	2
Ulceration					
% affected	0	18	12	6	0
Severity		1.6	1.7	1.7	
Dermal neoplasms					
% affected ***	0	8	64	98	6

* % mice affected
** Severity on a scale
1 = minimal
2 = mild
3 = moderate
4 = severe
*** % mice with neoplasms

The percent of mice with systemic neoplasms in the control and 81-08 animals was as follows:

Neoplasm	Group				
	Solvent control	Toluene control	BaP 0.01%	BaP 0.05%	81-08
Primary liver neoplasm					
Benign	4	2	6	0	4
Malignant	32	20	30	4	23
Primary lung neoplasm					
Benign	2	4	2	2	0
Malignant	0	0	0	0	0
Other neoplasms*					
Benign	0	2	0	0	2
Malignant	2	0	0	0	4
Total neoplasms	40	28	38	6	33
Group mean longevity**	103	100	96	61	108

* Includes malignant lymphomas that were observed in multiple sites, including treated skin
** Longevity shown in weeks

The data on dermal neoplasms that developed during the study are summarized in the following table

	Solvent control	Toluene control	BaP 0.01%	BaP 0.05%	81-08
% mice developing benign dermal neoplasm	0	0	0	4	4
% mice developing malignant dermal neoplasm	0	8	56	98	2
& mice with multiple tumors	0	0	28	60	0
Average tumors/mouse	0	.08	1	1.82	.06
No. mice with metastases	0	0	6	12	0
Mean latency (weeks)		111	86	49	113
Tumorigenic activity					
FEN *		43	49	47	46
% **		9	65	100	7

* FEN = No. of animals alive at the time of appearance of the median tumor plus any mice that died from tumor before that time OR when median latency is over 60 weeks, FEN = No. of animals alive at 60 weeks plus any mice that died with tumor before 60 weeks.

** % of mice developing tumors = No. developing tumors divided by FENx100

The tumor data of the 81-08 group were compared using a Chi square test with the data from the untreated and solvent controls and no significant differences were found.

Test substance : API 81-08 was applied undiluted.
The solvent control group was treated with toluene.
Benzo(a)pyrene was applied at concentrations of 0.01 and 0.05% in toluene.

12.11.2001 (9)

5.8 TOXICITY TO REPRODUCTION

: No data

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

: No data

(1) ABC Laboratories, Inc. (1998)
Static Renewal 48-hour Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Straight Run Naphtha, LSRN to Daphnia Magna. Study No. 43150.
Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri.

(2) ABC Laboratories, Inc. (1998)
Static Renewal 96 Hour Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Straight Run Naphtha, LSRN to a Freshwater Alga, Selenastrum capricornutum.
Project ID. 43151.
Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri.

(3) ABC Laboratories, Inc. (1998)
Static Renewal 96 Hour- Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Straight Run Naphtha, LSRN to Fathead Minnow (Pimephales promelas).
Project ID. 43152.
Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri.

(4) ABC Laboratories, Inc. Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri. 1998. Static Renewal 96 Hour- Method Validation For The Analysis Of The Water Accomodated Fraction Of Light Straight Run (LSRN) using Purge and Trap/Gas Chromatography. Project ID. 43155.

(5) American Petroleum Institute (1982)
Acute toxicity studies, sweetened naphtha Sample 81-08.
Study conducted by Hazleton Raltech.
API Medicine and biological science department Publication No. 30-31990, August 1982.

(6) American Petroleum Institute (1985)
Mutagenicity evaluation studies in the mouse lymphoma forward mutation assay, sweetened naphtha, sample 81-08.
Study carried out by Litton Bionetics Inc.
API Medical Research Publication No. 32-31233

(7) American Petroleum Institute (1986)
LC50 Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats API 81-08 Sweetened Naphtha CAS 64741-87-3
API Health and Environmental Sciences Dept. Publication No. 33-31827. June 1986

(8) American Petroleum Institute (1986)
Mutagenicity evaluation in the rat bone marrow cytogenetic assay, API 81-08, Sweetened naphtha (CAS 64741-87-3)
Study conducted by Litton Bionetics Inc.
API, HESD Research Publication 33-31093, April 1986.

(9) American Petroleum Institute (1989)
Lifetime dermal carcinogenesis bioassay of refinery streams in C3H/HEJ mice (A)-135R)
Study conducted by Primate Research Institute.
API HESD Publ. No. 36-31364.

(10) American Society for Testing and Materials (ASTM), 1991
Annual Book of ASTM Standards. Section 5, Petroleum Products, Lubricants and Fossil Fuels, ASTM, Philadelphia, Pa., 1991.

(11) CONCAWE (1992)
Ecotoxicological Testing Of Petroleum Products : Test Methodology.
Report 92/56,
CONCAWE, Brussels.

(12) CONCAWE (1995)
Fish - acute toxicity test: study no. 104858, test substance MRD-95-048.
Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels.

(13) CONCAWE (1995)
Physico-chemical characterization of gasoline samples, study no. 104990C. Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

(14) CONCAWE (1996)
Environmental risk assessment of petroleum substances: the hydrocarbon block method.
Report 96/52,
CONCAWE, Brussels.

(15) ECETOC (1998)
QSARS in the Assessment of the Environmental Fate and Effects of Chemicals. Technical Report No. 74

(16) ECETOC, (1996)
Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances.
Monograph 26.

(17) Harris, J.C. (1982)
Rate of Hydrolysis. In Handbook of Chemical Property Estimation Methods. p. 7-6. W. J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY, USA.

(18) King, R.W. et al., Skin carcinogenicity potential of petroleum hydrocarbons. 1 - Separation and characterization of fractions for bioassay. In: Applied Toxicology of Petroleum Hydrocarbons, pp. 123-138, American Petroleum Institute Publication, API, Washington DC, 1984.

(19) Mackay, D, A. DiGuardo, S. Paterson, & C. Cowan (1997)
EQC Model, ver. 1.01, 1997, available from the Environmental Modelling Centre, Trent University, Canada.

(20) Meylan, M, SRC 1994-1999.
LOGKOWWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(21) Meylan, M, SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(22) Springborn Laboratories, Inc. (1993)
CWE3 (Straight Run Gasoline) Toxicity to Freshwater Alga, Selenastrum capricornutum.
SLI Report # 93-6-4805.
Springborn Laboratories, Inc. Environmental Sciences division, 790 Main Street, Wareham, Massachusetts, USA.

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # OLEFINIC NAPHTHAS

Summary prepared by: American Petroleum Institute

Creation date: **20 DECEMBER 2000**

Printing date: **26 OCTOBER 2001**

Date of last Update: **10 DECEMBER 2001**

Number of Pages: **27**

NB. Reliabiliy of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : petroleum product
Physical status : liquid
Remark : Naphtha streams obtained from the catalytic cracking of heavy distillates into lighter fractions contain saturated, olefinic and aromatic hydrocarbons, mainly in the
range C_4 to C_{12} and boil in the range of approximately -20 to 230°C.

The streams that are rich on olefinic components are covered in this robust summary.

Information on several samples of olefinic naphtha streams is included here and their analytical characteristics are listed below.

	API [1] 83-09	LCCN [2]	LCCN-D [3] (vapor)
Parameter			
Gravity (°API)	69.5		
Sulfur (wt %)	0.02		
RVP (psia)	10.3		
IBP (°F)	87		
FBP (°F)	262		
Total Olefins (% by MS)	46.5	42.44	60.09
Total Naphthenes (% by MS)	-	9.14	2.66
Total Aromatics (% by MS)	9.0	15.78	1.85
Total Paraffins (% by MS)	44.5	29.77	35.14
n-Paraffins		5.32	7.11
Benzene (%)	1.2	1.21	0.79
Carbon No. (vol%)			
4		0.44	1.55
5		24.65	62.35
6		28.22	28.50
7		22.52	6.22
8		16.07	1.27
9		6.85	0.10

[1] API 83-09 is a sample of LCCN
[2] LCCN is Light Catalytically cracked Naphtha
[3] LCCN-D is a distillate of LCCN

26.10.2001

2.2 BOILING POINT

The olefinic naphtha streams boil in the range of approximately minus 20 to 230 °C (See section 1.1)

2.5 PARTITION COEFFICIENT

Log pow	:	2.13 - 4 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65.
Year	:	2000
GLP	:	No
Test substance	:	Light Catalytically Cracked Naphtha
Remark	:	Log P values represent the spread of calculated and/or measured values for the C_5 to C_9 hydrocarbon components found in LCCN (CAS No. 64741-55-5). Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCCN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.
Reliability	:	(2) Valid with restrictions

26.06.2001 (11)

2.6.1 WATER SOLUBILITY

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	yes
Test substance	:	Light Catalytically Cracked Naphtha
Method	:	Water Accommodated Fractions (WAFs) of LCCN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 72 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of selected components indicated freshwater and saltwater solubilities of 4.6 and 4.3 ppm respectively. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of benzene, toluene, ethylbenzene, o-xylene and p-xylene, which represent 13% composition of the test substance. Concentrations for these components reached equilibrium in freshwater and saltwater by 24 and 12 hours respectively.
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LCCN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) Valid with restrictions

26.10.2001 (3) (4) (6) (7) (18)

3.1.1 PHOTODEGRADATION

Type	:	Calculation
Light source	:	Sun light
Rel. intensity	:	Based on Intensity of Sunlight
Sensitizer	:	OH
Rate constant	:	cm^3/(molecule*sec)
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	no
Test substance	:	Light Catalytically Cracked Naphtha
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half-lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in LCCN CAS No. 64741-55-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCCN sample. Based on a 12-hour day, the range for atmospheric half-lives for LCCN constituents due to OH reactions is: 1.44 hours (1-methyl cyclopentene) to 15.985 days (isopentane). The range for atmospheric half-lives due to O_3 reactions for LCCN olefinic constituents (accounting for approximately 30% composition) is 38.378 min (1-methyl cyclopentene) to 22.920 Hrs (C_5 olefins).
Result	:	Indirect photolysis: Sensitizer: OH radical Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate constant: 0.6691E-12 cm^3/mol-sec (isopentane) to 89.41 E-12 (1-methyl cyclopentene) Half life: 1.44 hours to 15.985 days Sensitizer: O_3 radical Conc. of sensitizer: 7E1103/cm^3 Rate constant: 1.2 E-17 to 43-17 cm^3/molecule-sec Half life: 38.378 min to 22.920 Hrs.
Reliability	:	(2) valid with restrictions

26.06.2001 (12)

3.1.2 STABILITY IN WATER

Test substance	:	Light Catalytically Cracked Naphtha
Conclusion	:	Hydrolysis unlikely
Reliability	:	(1) Valid without restriction

26.06.2001 (8)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	Calculated
Media	:	soil, air, water, suspended sediment and sediment
Method	:	Calculated according to Mackay Level 1
Year	:	2000
Remark	:	Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_9 hydrocarbon components found in LCCN, CAS No 64741-55-5. Detailed

hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCCN sample.

The majority of LCCN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals and ozone.

Result :

Medium	% distribution
Air	97 to 100
Soil	0.00 to 1.2
Water	0.01 to 2.7
Sediment	<0.001 to 0.02
Suspended sediment	<0.001 to 0.02

Conclusion : This complex petroleum mixture is expected to partition primarily to air.
Reliability : (2) Valid with restrictions
05.10.2001 (10)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Mixed, adapted inoculum of domestic activated sludge and soil
Contact time : 56 day
Result : Inherently biodegradable
Method : CONCAWE test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year : 1999
GLP : Yes
Test substance : Light Catalytically Cracked Naphtha
Method : Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO_2 production. By day 28 approximately 74% of the test material was degraded, then essentially reached a plateau in degradation rate until day 56. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63% actual) was observed by day 14, and total blank CO_2 production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21 °C, which deviated from the protocol value of 22 ±2°C. This deviation was not expected to have affected the outcome of this study.

	% Degradation (sd)	
Test Day	Hexadecane	Test Material
3	13.93 (1.85)	16.83 (9.56)
7	34.40 (4.54)	30.99 (0.56)
14	63.17 (0.94)	51.66 (3.33)
21	77.26 (6.52)	54.82 (6.24)
28	90.35 (7.14)	74.30 (1.24)
35	85.13 (n=1)	65.02 (1.37)
42	85.21 (n=1)	74.82 (0.54)
49	96.93 (8.94)	70.78 (6.48)
56	94.69 (4.10)	79.22 (12.28)

Test condition : Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two-week adaptation period. Triplicate test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass-distilled water and mineral salts (phosphate buffer, ferric

chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of soil was obtained at a depth between 5-10cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substance was added directly to test systems using a 10 µl Hamilton gas tight syringe. The volume required to achieve the specified mg carbon/L concentrations were calculated based on %carbon and specific gravity of the respective substance. The test substance %carbon (0.8724) and specific gravity (0.7220 mg/µl) information was supplied by the Sponsor. Hexadecane %carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/µl) was obtained from Verschueren (1983). Addition of respective substance was performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 °C (± 2°C) in the dark.

Biodegradation by CO_2 determination-test initiation and procedure. On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume(100 ml inoculum/l).
Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/l concentration.
Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO_2 analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22 °C (± 2°C).

On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc. H_3PO_4 was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, and then analyzed for CO_2 using gas chromatography-thermal conductivity detection. Quantitation of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability : (1) valid without restriction
26.10.2001 (14)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	96 Hr Static Acute Toxicity Test w/ Daily Renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year	:	1995
GLP	:	Yes
Test substance	:	TS: Light Catalytically Cracked Naphtha
Method	:	Statistical Method: (FT - ME) LL_{50} and LC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 0, 1, 0, 0, 4 and 20, respectively 0, 3.0, 7.4, 15, 37 and 74 mg/l treatments. All surviving organisms exhibited normal behavior. 96-hr LL_{50} = 46 mg/l, 37-74 mg/l w/ 95% C.I. (as nominal loading rate) 96-hr LC_{50} = 4.1 mg/l, 3.2-7.0 mg/l w/ 95% C.I. (measured concentrations)
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was Mobil Technical Center well water. Nominal loading rates of 0, 3.0, 7.4, 15, 37 and 74 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a Teflon lined neoprene stopper housing two Teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, Para film was used to seal the external joint between the neoprene stopper and glass bottle. After stirring for 24 hrs using 25%or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-PID for concentrations of the following: benzene, toluene, ethylbenzene, and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes. Fish were hatched and raised in-house, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8L glass containers with Teflon lined caps. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving adequate volume to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Water temperature was 21.4-21.8 °C. Test photoperiod was 16 hrs. light

and 8 hr dark. Dissolved oxygen measurements were between 5.2 and 8.6, pH values between 7.61 and 8.2.

Reliability : (2) Valid with restrictions
NOEC values not reported as sublethal effects and moderate mortality (20%) were observed at the 37-ppm (nominal loading) treatment, which is reported to be the NOEC.

26.10.2001 (15)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : 48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species : Daphnia magna (Crustacean)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1995
GLP : yes
Test substance : TS: Light Catalytically Cracked Naphtha
Method : Statistical Method: (FT - ME) EL_{50} and EC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result : Mortality (no. of deaths/treatment) at 48 hrs: 0, 0, 0, 20, 20 and 20 for 0, 6.4, 13, 25, 51 and 102 mg/l treatments.

48-hr EL_{50} = 18 mg/l (95% C.I. 13 to 25 mg/l) based upon nominal loading rate.
48 hr EC_{50} was 1.4 ppm (95% C.I. 0.99 to 1.95 mg/l); based on total measured concentrations.

48-hr NOEC = 13 mg/l based upon nominal loading rate
48 hr EC_{50} was 0.99 ppm based on total measured concentrations.

Test condition : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was aged well water.

WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 1.2L of water for 24 hr in aluminum foil covered 1 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25%or less vortex, the contents of the aspirator bottles were allowed to settle for approximately 45 minutes, then drained from the port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes.

Range finding toxicity studies were conducted at 1.3, 10 and 102 mg/l loading, using WAFS, which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 6.4, 13, 25, 51 and 102 mg/l loading, using WAFS, which were divided into duplicate aliquots and tested.

Test vessels were teflon cap-sealed 265 ml glass jars with10 daphnids per jar and were completely filled with test solution.

During the study test system solutions: dissolved oxygen concentration

range: 8.0 to 8.6; pH ranged from 7.94 to 8.40; temperature was 19.1 to 20.2 Deg C; hardness (mg/l) ranged from 172 - 180; alkalinity (mg/l) was 124-132 and conductivity (umhos) values were 360 - 405.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1994. The primary culture was obtained from Aquatic Research organisms, Hampton, NH, which was derived from EPA laboratory culture, in Cincinnati, Ohio.

Reliability : (2) valid with restrictions
Measured concentrations represent only 13-20% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

26.10.2001 (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : EPA. 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-8, ES-5.
Year : 1995
GLP : yes
Test substance : Light Catalytically Cracked Naphtha
Method : Statistical Method: LL_{50} and LC_{50} calculated using probit analysis. ASTM Special Technical Publication 634. 1977, pp 65-84. All NOEL/NOEC values calculated using Fisher's exact test.
Result : Percent inhibition on growth determined by cell density (cells/ml):
96 hour EL_{50}=64 mg/l (44-111 mg/l CI @95%)
96 hour EC_{50}= 4.6mg/l (2.9-8.8 mg/l CI @95%)
96 hour NOEL=51 mg/l
96 hour NOEC=3.5 mg/l
Subcultures placed in fresh media (no test substance) after acute testing for six days indicated that growth inhibition was algistatic in all treatments, with the exception of the 102 ppm, which was determined to be algicidal. No excursions from the protocol were noted. However, range finding and two previous definitive tests were performed and considered inconclusive due to inconsistencies in control and treatment cell densities, which presumably were resolved by modification of the AAP media. Additionally, control growth showed a lag during the first 72 hours of the study.

Nominal (mg/l) Conc. (meas.mg/l).	96hr cell density (Cells/ml)	(% Inhibition)
Control	8.4 x10^3	na
6.4 (0.093)	3.2 x10^4	- 281.1
13 (0.130)	9.73x10^3	- 16.0
25 (0.429)	1.99x10^4	-136.9
51 (1.87)	1.36x10^3	53.0
102 (4.85)	2.59x10^3	69.2

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 4.4L of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, pH adjusted to 7.5 + 0.1 with 0.1NHCl and sterilized by 0.22 micron filtration) in 4.0 liter aspirator bottles. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with Teflon coated stir bars for approximately 24 hours at room temperature in a hood darkened with aluminum foil. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were

125ml glass Erlenmeyer flasks that were completely filled (140 ml) with treatment solution and inoculated with algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media, and transferred every 4-8 days to fresh media. Original algal cultures obtained from American Type Culture Collection (ATCC Strain 22662), Rockville, MD, September 1995. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 6.4, 13, 25, 51 and 102 mg/l The initial algal concentration was 1.0×10^3 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 400 ± 50-ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0,24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, o-xylene and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes.

Test temperature was 24 ±2 °C. The pH was 7.5 at test initiation, pH value at test termination not included in report.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

26.10.2001 (17)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Undiluted
Value	:	> 5000 mg/kg bw
Year	:	1986
GLP	:	Yes
Test substance	:	LCCN (Sample API 83-20)
Method	:	Groups of five male and five female fasted rats were given API 83-20 as a single oral dose of 5 g/kg. The animals were then allowed food and water *ad-libitum* and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded.
Result	:	There were no mortalities during the study. Body weights had increased by day 7 after dosing and further increases were recorded 14 days after dosing. Clinical signs of toxicity were observed during the 24 hours immediately after dosing and appeared normal thereafter. Clinical signs included: hypoactivity, ataxia, diarrhea, lacrimation, yellow-stained anal area, excessive salivation and respiratory congestion. There were no treatment-related lesions observed at necropsy.
Conclusion	:	Oral LD_{50} was greater than 5 g/kg for males and females.
Reliability	:	(1) Valid without restriction

26.10.2001 (1)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Air
Exposure time	:	4 hour(s)
Value	:	> 5.3 mg/l
Year	:	1987
GLP	:	Yes
Test substance	:	LCCN (Sample API 83-20)
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-20 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed and subjected to a gross post-mortem examination. For all animals, including those found dead during the study, the lungs were removed,

fixed and examined histologically.

Result : The mean analytical exposure concentration was measured and found to be 5.28 ±0.55 mg/l.
Gravimetric samples, collected on glass fiber filters suggested little or no aerosol in the chamber.
Most animals exhibited languid behavior and squinted eyes during the second hour of the exposure. Polypnea was observed in all animals when removed from the chamber at the one-hour post exposure observation period. Rhinorrhea was exhibited by two animals on day two of the test. All animals appeared normal subsequently and there were no mortalities during the study.
With the exception of one animal (female) all animals had body weights that were considered unremarkable.
There were no remarkable gross or microscopic findings.

Reliability : (1) valid without restriction
26.10.2001 (2)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 4
Vehicle : Undiluted
Value : > 3000 mg/kg bw
Year : 1986
GLP : Yes
Test substance : LCCN (Sample API 83-20)
Method : The skin of the patched area of four rabbits of each sex had been abraded whilst the other four had intact skin.
A weighed quantity of undiluted test material was applied to the dorsal skin of each of 4 male and 4 female rabbits at a dose level of 2.0 g/kg. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later and the animals were observed for a total of 14 days post-dosing.
At study termination, the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Since there were 2 mortalities recorded in this study, the experiment was repeated using a dose level of 3 g/kg.

Result : A pain response was elicited from all animals during application of the test material to the skin. Dermal irritation ranging from slight to severe was observed for erythema and slight to marked for atonia, desquamation, fissuring and coriaceousness.
At the 2 g/kg dose level, clinical observations included: diarrhea, anorexia and hypoactivity and one female in this dose group died on day 7 following dosing.
There were no clinical signs of toxicity in the 3 g/kg dose group and no animals in this group died.
At necropsy, skin lesions were observed more frequently in the 2 g/kg group than the 3 g/kg group.

Conclusion : LD_{50} was greater than 3 g/kg for both male and female rabbits.

Reliability : (1) Valid without restriction
26.10.2001 (1)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Remark : Not relevant
26.10.2001

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : 3.7
Result : Moderately irritating
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : LCCN (Sample API 83-20)
Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. One area was intact and the other abraded skin.
The treated area was then covered with an occlusive dressing.
After 24 hours, the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 96 hours, 7 and 14 days. Results of the 24 and 72-hour readings were used to determine the Primary Irritation Index.
Result : The scores for erythema and edema were marginally greater for abraded skin than intact skin, but the difference was not biologically significant.
Scores for abraded skin at each of the observation intervals were:

Time	Erythema	Edema	Irritation score*
24 hours	1.8	2.0	3.5
72 hours	2.3	1.7	3.8
96 hours	1.5	1.5	2.6
7 days	1.2	0.2	1.2
14 days	0.0	0.0	0.0

* Irritation score calculated as the sum of irritation scores for each test site divided by the number of animals at each observation period.
PII is the sum of the 24- and 72- hour total irritation scores divided by 2

Reliability : (1) Valid without restriction
26.10.2001 (1)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted

Dose	:	.1 ml
Comment	:	Other: See method
Number of animals	:	9
Method	:	Draize Test
Year	:	1986
GLP	:	Yes
Test substance	:	LCCN (Sample API 83-20)
Method	:	0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits; the other eye was untreated and served as control. After 20 to 30 seconds, the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.
Result	:	No pain response was elicited from any of the animals when the test material was applied to the corneal surface. The primary eye irritation score (=total eye irritation score for all animals divided by the number of animals) was 1.0 after 1 hour for those animals with unwashed eyes compared to 3.3 for those whose eyes had been washed. An irritation score of zero was recorded at all other times. Neither iridial nor corneal irritation resulted from application of the test material.
Reliability	:	(1) Valid without restriction

26.10.2001 (1)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	Induction undiluted occlusive epicutaneous Challenge 25 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	Paraffin oil
Result	:	Not sensitizing
Classification	:	Not sensitizing
Year	:	1986
GLP	:	Yes
Test substance	:	LCCN (Sample API 83-20)
Method	:	0.4 ml of undiluted test material was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residue of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application, a challenge dose (0.4 ml of a 25% mixture in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream. Positive control (2,4-dinitrochlorobenzene, as a 0.3% w/v

solution in 80% aqueous ethanol), vehicle control (paraffin oil) and naive control groups were included in this study and the procedure for these was the same as for the test groups.

Result : No skin reactions were observed following the application of the challenge dose in either the naive controls or the group that had been exposed to test material. Scores of 0.2, 0.3 and 0.5 for erythema were recorded for the paraffin oil controls.

In contrast, all positive control animals developed a skin response following the challenge procedure.

Reliability : (1) Valid without restriction
26.10.2001 (1)

5.4 REPEATED DOSE TOXICITY

Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	15 weeks
Frequency of treatment	:	6 hours/day, 5 days/week
Post obs. period	:	4 weeks
Doses	:	Target: 750, 2500 & 7500 ppm. Actual: 756, 2507 & 7533 ppm
Control group	:	Yes
Method	:	EPA OTS 798.2450
Year	:	2001
GLP	:	Yes
Test substance	:	LCCN-D (Distillate of LCCN)
Method	:	Groups of 16 male and 16 female rats underwent whole body exposures to 750, 2500 and 7500 ppm LCCN-D. Exposures were for 6 hours each day, 5 days per week, for at least 65 exposures, over a period of 15 weeks. Extra groups of 16 rats of each sex were exposed to the high dose level and for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 15 weeks exposure. Neurobehavioral evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14/15 and after the 4 week recovery period for the recovery animals. Animals were not exposed to LCCN-D during these tests. Following 15 weeks of exposure, 16 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically. At the end of the 4-week recovery period, 16 animals of each sex from the high and control groups were necropsied and selected tissues were examined microscopically. During the study, clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 15 weeks and 20 weeks (recovery groups). Body weights and food consumption were measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14

and 18 weeks for hematological and clinical chemical measurements.

At termination (after 15 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. 10 animals of each sex were designated for non-neuropathological examination and 6 of each sex for neuropathological examination.
For the non-neuropathology animals, the following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) was removed from the control and high dose animals and were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination.
Animals designated for neuropathological examination were subjected to a detailed examination of central and peripheral nervous tissues.

Neurobehavioral studies were undertaken as follows:

Motor activity
Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 15 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery
An assessment of the following was made:

Home cage evaluations for Posture, vocalization, palpebral closure.

Handling evaluations for reactivity to general stimuli, signs of autonomic function.

Open field behavior: arousal level, gait, urination and defecation frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.

Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result : No exposure-related clinical observations were noted either during exposure or during non-exposure periods and no ocular abnormalities were observed.
Although the males in the high dose group were slightly lighter than the controls, the difference was not significant. In the females however, the difference was statistically significant. At the end of the 4-week recovery period body weights of the high dose males and females were comparable to the corresponding controls. During the 4-week recovery period, the high dose males and females had food consumption that was greater

(statistically significant) than controls.
At 15 weeks, the following hematological changes were recorded.

7500 ppm males
Decreased hemoglobin concentration (8%)
Decreased hematocrit (7%)
2500 ppm males
Decreased MCHC (3%)
7500 ppm females
Decreased MCHC (4%)

After the 4-week recovery period, all hematological values were considered normal.

At 15 weeks, there were no abnormal clinical chemistry values. After the 4-week recovery period however, glucose and albumin was raised in the 7500-ppm females by 21 and 15% respectively. Since the values were within the normal range, they were not considered toxicologically significant.

Neurobehavioral studies
There was no evidence of any effect on motor activity either after 15 weeks exposure or after the 4-week recovery period.
There was no evidence of a treatment-related effect in the functional operational battery that was carried out.

Pathology
With the exception of those listed below, absolute and relative organ weights were not affected by treatment.

		Group	
Parameter	2500 ppm	7500 ppm	Recovery
MALES			
Abs Kidney		21% up	
Rel Kidney	15% up	32% up	
Rel Liver		23% up	
FEMALES			
Rel Kidney		18% up	
Abs Liver			24% up
Rel Liver		12% up	
Rel Brain			9% down

There were no microscopic findings in either the liver or brain of the groups in which organ weight changes had been recorded.

The only treatment-related microscopic changes were found in the kidneys and nasal turbinates as follows.

Nasal turbinates
Goblet cell hypertrophy/hyperplasia and hyperplasia of the respiratory epithelium were seen in a number of animals from all groups. Based on the incidence and/or severity both findings were slightly more pronounced in the 7500 ppm group and were seen primarily in the anterior region of the nasoturbinal tissues. At the end of the 4-week recovery, the incidence and severity of goblet cell hypertrophy/hyperplasia and hyperplasia of the respiratory epithelium were considered to be similar in high dose and control animals. In the 7500 ppm females severity of the findings in the nasoturbinal tissues was slightly less after recovery than at the end of the

15 week exposure. In the males the severity and incidence of the effects was the same in the nasoturbinal tissues after the recovery period as it was after 15 weeks exposure. These findings are considered indicative of exposure to a mild irritant.

Kidney
At the end of 15 weeks exposure intracytoplasmic eosinophilic/hyaline droplets were seen in the epithelium lining the proximal convoluted tubules of the kidneys. It was seen in several males of the control and 750 ppm group and all males in the 2500 and 7500 ppm groups. At the end of the 4-week recovery period the incidence and severity in controls and 7500 ppm males was similar.
Several males from the 7500 ppm group and one from the 2500 ppm group had cortico-medullary tubules which were dilated and contained granular casts. None of these findings were found in the females and furthermore, none were observed at the end of the 4 week recovery period.
Subacute/chronic interstitial inflammation was seen in several rats from all groups, except the control group males and the 7500 ppm females. In the males from the exposure groups, the incidence showed a dose-related increase. Basophilic convoluted tubules were seen in a small number of males from the 2500 and 7500 ppm groups.

At the end of the 4-week recovery period there was an indication of some reversibility of the kidney effects.

In the post exposure animals, brain length and width measurements showed no test-material-related effects.

Reliability : (1) Valid without restriction
26.10.2001 (9)

Species : Rat and Mouse
Sex : Male/female
Strain : Rat - Sprague Dawley, Mouse - CD-1
Route of admin. : Inhalation
Exposure period : 13 weeks
Frequency of treatment : 6 hours/ day, 5 days/week for 13 weeks
Doses : Target: 500, 2000 & 8000. Actual: 530, 2060 & 7690 mg/m3
Control group : Yes
Year : 1996
GLP : No data
Method : Groups of 10 rats of each sex and 10 mice of each sex were individually housed in inhalation chambers. The rats and mice underwent whole body exposures to LCCN vapors. Exposures were for 6 hours/day, 5 days/week for approximately 13 weeks at nominal concentrations of 500, 2000 or 8000 mg/m^3.
Extra groups of 10 rats and mice of each sex served as sham and untreated controls.
Food and water was available ad-lib, except during the exposure periods.
Clinical observations were made regularly and body weights were recorded weekly.

At the end of the 13 weeks exposure, the rats were fasted for 16 hours before blood samples were taken for hematological and clinical chemical measurements.
All animals were then sacrificed and necropsied. Organs were weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet and dry weights of the right apical and right middle lung lobes were also recorded. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to

determine the number of testicular spermatids.

The following tissues from the high dose and sham treated animals were examined histologically: adrenals, kidney, bone and marrow (sternum), pancreas, brain, submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach, colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver,
tracheobronchial lymph nodes, lung (left lobe), nasal
turbinates, muscle, urinary bladder, sciatic nerve, and any gross lesions. Additional sections included lung from untreated controls and kidney from 0, 2060 and the 7690 mg/m^3 exposure groups.

Result : No treatment-related changes were observed in either species in clinical signs, body weight, clinical chemistry or hematology except four male rats in the high dose group that had lesions on the skin in the scrotal area. This was attributed possibly to an interaction between abrasions of the skin against the floors of the cages and the exposure to the high concentrations of LCCN.

Organ weights were unaffected in either species, except for uterus weights. Uterine weights in the rats were less than untreated controls for all exposed groups, but not less than the sham controls. The actual weights (g) shown below, were not considered to be related to LCCN because they were not dose-related, and there was no difference between the sham and untreated controls. Additionally, no similar effect was observed in the mice.

Untreated controls	0.69 +/- 0.17
Sham controls	0.62 +/- 0.07
530 mg/m^3	0.55 +/- 0.12
2060 mg/m^3	0.52 +/- 0.05
7690 mg/m^3	0.54 +/- 0.09

No treatment-related abnormalities were observed in any of the organs examined microscopically. The incidence of the occurrence of hyaline droplets in dilated tubules was similar in the controls and the high dose males and was not considered relevant.

The number of sperm per gram of cauda epididymis was significantly lower in the 7690 mg/m^3 group than in the sham controls but not the untreated controls. The number of epididymal sperm was not significantly affected by exposure. In addition, the number of testicular spermatids and the percentage of abnormal sperm in the cauda epidiymis were not affected by exposure to 7690 mg/m^3 compared to either control group.

Test substance : LCCN CAS # 64741-55-5
Vapors of LCCN were generated in a glass countercurrent generator (one for each concentration).
As liquid LCCN flowed down the coil, nitrogen passed upwards and carried off vapors of the more volatile components. Mainstream air was used to dilute the vapor to the required concentration.

Vapor concentration was monitored at approximately hourly intervals during each exposure period.

Concentrations (Target and actual) are shown below.

Target (mg/m^3)	Actual (mg/m^3)
500	530 +/ 90
2000	2060 +/- 250
8000	7690 +/- 730

In addition the composition of neat LCCN (liquid), its static headspace and the inhalation chambers was assessed. The results shown below confirm that the animals had been exposed to the lighter components of LCCN .

Component	% in Liquid LCCN	Static Headspace	Exposure Chamber
Total C_4/C_5 nonaromatics	18.6	76.5	38.2
n-Butane	0.2	5.5	
Isobutane	-	1.0	0.2
Butenes	1.7	11.4	
n-Pentane	1.4	3.3	7.0
Pentenes	3.8	13.1	
Methyl butene	5.2	11.9	
Total C_6 nonaromatics	16.2	14	
n-Hexane	1.0	0.9	
2,3-Dimethylbutane	1.3	1.0	
2-Methylpentane	4.1	6.0	12.2
3-Methylpentane	2.5	2.7	7.5
Methylpentene	1.7	0.9	
Hexenes	2.1	1.1	
Methylcyclopentane	2.3	1.8	
4-Methylcyclopentane	0.6	0.5	
Total C_7 nonaromatics	10.2	1.9	
Total C_8 nonaromatics	8.0		
n-Octane	0.3		
2,2,4-Trimethylpentane	0.8	0.4	2.7
Total Aromatics	24.0		
Benzene	0.1	0.6	2.3
Toluene	4.6	0.8	4.7
Ethylbenzene	1.5	0.1	
Xylenes	7.6	0.5	2.3

Reliability : (2) Valid with restrictions
The data presented in the publication are more or less limited to those showing effects. Where no effects are reported, actual data are not shown. Nevertheless, the study is sound and helpful in assessing the effects of LCCN light ends on this biological endpoint.

26.10.2001 (5)

5.5 GENETIC TOXICITY 'IN VITRO'

Remark : No data
26.10.2001

5.6 GENETIC TOXICITY 'IN VIVO'

Remark : No data
26.10.2001

5.7 CARCINOGENITY

Remark : No data
26.10.2001

5.8 TOXICITY TO REPRODUCTION

Type	:	Reproductive/developmental toxicity screening study, (OECD 421)
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Frequency of treatment	:	6 hours/day, 7 days/week
Premating exposure period		
Male	:	2 weeks
Female	:	2 weeks
Duration of test	:	
Doses	:	Target: 750, 2500 & 7500 ppm. Actual: 752, 2512 & 7518 ppm
Control group	:	Yes
NOAEL Parental	:	2500 ppm
other: NOAEL for reproductive performance and developmental toxicity	:	7500 ppm
Method	:	OECD combined repeated dose and reproductive/developmental toxicity screening test
Year	:	1999
GLP	:	Yes
Test substance	:	TS: LCCN-D
Method	:	Groups of 10 rats of each sex were exposed to 750, 2500 or 7500 ppm. LCRN-D for 6 hours /day, seven days/week. A group of 10 rats of each sex served as sham treated controls. Parental females were exposed for 14 consecutive days prior to mating, throughout mating and days 0-19 of gestation. Dams and their litters were sacrificed on post-partum day 4. Unmated females and parental males were exposed to the test material for 14 days prior to mating, throughout mating and for 23 additional days following completion of the mating period. These animals were sacrificed shortly after the last litters were delivered reached post-partum day 4. Mating Within each group one male was co-housed with the same female until evidence of mating was observed (presence of sperm in vaginal smear or copulatory plug). The day of mating was designated day 0 of gestation. Following mating, the females were housed individually and continued their exposures to test material until day 19 of gestation. Females not showing evidence of mating following a 14-day mating period continued their exposures. If such a female showed signs of being pregnant, it was removed from the exposure regimen and observed for parturition. Observations All parental animals were regularly observed for mortality and gross pharmacologic signs. A physical examination, including palpation for tissue masses was carried out daily 30 mins after removal from the exposure chambers. Body weights and food consumption were measured throughout the study. From day 20 of gestation, females (pregnant and non-pregnant) were observed for signs of parturition. As soon as possible after delivery, litters were observed for the number of live and dead pups and for any

abnormalities. Litters were also observed twice daily for unusual findings and dead pups.
On days 0 and 4 of lactation, the pups were counted, weighed and their sex was determined by external observation.

Pathology
Males were killed as a group shortly after the last litters delivered had reached day 4 of lactation.
Females with litters that reached day 4 of lactation were killed the next day or shortly thereafter.
Unmated females and those that did not deliver were killed 23 days after completion of the mating period.
At post mortem, a complete macroscopic examination was carried out on all adult animals. The following organs were weighed and organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, lung, spleen, epididymes, testes and thymus. Post mortem examination of females included a count of uterine implantation scars when present.

Pups were sacrificed on day 4 of lactation and underwent a complete macroscopic examination and a determination of sex by internal examination. All pups were preserved with viscera intact. Pups found dead at birth and that died prior to day 4 of lactation also underwent a gross external and internal examination. Dead pups were not eviscerated, but were preserved intact.

27 tissues were preserved from all adult animals in all dose groups. Ovaries, testes, epididymes, nose with nasal turbinates, and any grossly observed abnormalities were processed and sections examined histologically for all males and female parental animals in the control and highest dose group. Four sections were prepared and examined microscopically of the skull containing the nasal turbinates. These were

area between upper incisor and incisive papilla
area between incisive papilla and first palatal ridge
area between second palatal ridge and first upper molar
area between first upper molar and nasopharynx.

Result : All animals survived to scheduled sacrifice. Red staining on the snout was seen with increasing frequency in the mid and high dose animals of both sexes throughout the study. Microscopic examination of the nasal turbinates of the sham-exposed and high dose animals did not reveal any significant changes.

Although all treated groups gained slightly less weight than the sham treated controls, the differences were not statistically significant. Food consumption was comparable in all groups. Apart from those listed below, absolute and relative organ weights were unaffected by treatment.

High dose Males
Absolute kidney weight increased (18%)
Relative kidney weights increased (24%)
Relative liver weights increased (15%)

High dose females.
Absolute spleen weights increased by (19%)
Relative spleen weights increased by (19%)

At necropsy, no organs appeared abnormal.

Microscopic examination of kidneys from one high dose male with a dilated

renal pelvis at necropsy revealed hyaline droplet formation and tubular dilatation of tubules in the cortico-medullary junction. This finding is consistent with male-rat-specific light hydrocarbon nephropathy.
No test-related microscopic changes were observed in the testes or epididymes of adult male rats or ovaries of adult female rats in the high dose group.

Reproductive/fertility effects

All groups had a fertility index of >90% and all groups had a live birth index greater than or equal to 98%.
Data are summarized below.

Parameter	Dose group (ppm)			
	0	750	2500	7500
Females on study	10	10	10	10
Litters with liveborn	9	8	9	10
Implantation sites	155	126	139	160
Mean	17.2	15.8	15.4	16
Pups delivered (total)	149	110	132	152
Liveborn	149	108	131	151
Live birth index (%)	100	98	99	99
Pups dying				
Day 0	0	2	1	1
Days 1-4	4	2	2	1
Pups surviving 4 days	145	106	129	150
Viability index (%)	97	98	99	99
pup sex distribution				
Day 0 M/F (ratio)	72/77	50/58	65/66	87/64
Day 4 M/F (ratio)	72/73	49/57	65/64	87/63
Pup weight/litter (g)				
Day 0	6.3	6.6	6.4	6.4
Day 4	9.9	10.8	10.1	10.3

External and internal examination of pups sacrificed on day 4 of lactation were unremarkable.

Reliability : (1) Valid without restriction
26.10.2001 (13)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : During days 0 to 19 of gestation inclusive.
Frequency of treatment : 6 hours each day
Doses : Target: 2000 & 8000 mg/m^3. Actual: 2150 & 7660 mg/m^3
Control group : Yes
Year : 1996
GLP : No data
Test substance : LCCN CAS # 64741-55-5
Method : Four groups of 15 presumed-pregnant female rats were assigned to the following groups: Untreated controls, sham-treated controls, 2000 and 8000 mg/m3 test material.
Exposures were for 6 hours each day on days 0 to 19 of gestation.
All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation.
On day 20, each female was sacrificed and all organs were examined

grossly. Serum samples were analyzed for a variety of parameters, including serum iron and lactic dehydrogenase.
The number of corporea lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early resorptions and live and dead fetuses recorded. Each fetus was identified for its sex, was weighed and the crown-rump distance was measured. Each fetus was examined for external anomalies. Half the fetuses were fixed in Bouin's solution and examined for visceral anomalies and the remaining fetuses were prepared for examination for skeletal anomalies.

Result **:** There were no treatment-related clinical abnormalities or differences in body weight.
Results of the reproductive parameters are listed below.

			LCCN	
Parameter	No treat	Control Sham treat	2150	7660
Females mated	15	15	15	15
Females pregnant	14	13	14	15
Corpora lutea	18	18	16	18
Implantation sites	16	16	14	16
Primplantation loss (&)	10	12	14	8
Viable fetuses/litter	15	14	14	15
Resorptions	0.7	0.6	0.8	1.7 *a
Resorptions (%)	4.6	3.9	4.7	10.4 *a
Dams with resorptions	9	5	8	13 *b

*a Significant difference from untreated and sham treated controls

*b Significant difference from sham treated controls

It is clear that with the exception of resorptions, no other parameter was affected by exposure.

During the external examination of fetuses, a sham treated animal had gastroschisis and one fetus from the 2150 mg/m^3 group had a tail that was short and filamentous.

Fetal body weights and crown-rump lengths were unaffected by treatment.

No visceral abnormalities were observed.

There was an increased number of skeletal variations in animals housed in the exposure chambers (exposed and sham treated controls) when compared to the untreated controls.
The authors concluded that these alterations were not related to LCCN since they occurred at the same incidence in the sham treated controls as well.

The findings are tabulated below.

Parameter	No treat	Control Sham treat	LCCN 2150	7660
Caudal vertebrae: transverse process incompletely ossified	18(16)	42(40)	41(40)	45(39)
Sacral vertebrae transverse process incompletely ossified	7(6)	23(22)	17(17)	28(24)
Incompletely ossified sternebrae	83(75)	80(76)	91(89)	101(88)

Test substance : A description of atmosphere generation is given in a publication by the same authors in section 5.4.

Actual concentrations in this study were:
2150 +/- 260 and 7660 +/- 570 mg/m^3.

Reliability : (1) valid without restriction
26.10.2001 (5)

(1) American Petroleum Institute (1986)
Acute oral toxicity study in rats, acute dermal toxicity study in rabbits, primary dermal irritation study in rabbits, primary eye irritation study in rabbits, dermal sensitization study in guinea pigs.
Study conducted by Hazleton Laboratories Inc.
Health and Environmental Sciences Dept. Publ. No. 33-32722

(2) American Petroleum Institute (1987)
Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats API 83-20 Light catalytic cracked naphtha (CAS 64741-55-5).
Study conducted by Hazleton Laboratories America Inc.
Health and Environmental Sciences Dept. Publ. No. 34-32777

(3) CONCAWE (1992)
Ecotoxicological Testing Of Petroleum Products : Test Methodology.
Report 92/56,
CONCAWE, Brussels.

(4) CONCAWE (1996)
Environmental risk assessment of petroleum substances: the hydrocarbon block method.
Report 96/52,
CONCAWE, Brussels.

(5) Dalbey, W. E., Feuston, M. H., Yang, J. J., Kommineni, C. V and Roy, T. A. (1996)
Light Catalytically cracked naphtha: subchronic toxicity of vapors in rats and mice and developmental toxicity screen in rats.
J. Toxicol. and Env. Health Vol 47, pp 77-91

(6) ECETOC (1998)
QSARS in the Assessment of the Environmental Fate and Effects of Chemicals. Technical Report No. 74

(7) ECETOC, (1996)
Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances.
Monograph 26.

(8) Harris, J.C. (1982)
Rate of Hydrolysis. In Handbook of Chemical Property Estimation Methods. p. 7-6. W. J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY, USA.

(9) Lapin, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Roth, R., Schreiner, C., White,R., Mandella, R. and Hoffman, G. (2001)
Toxicity evaluation of petroleum blending streams: Inhalation subchronic toxicity/neurotoxicity study of a light catalytic cracked naphtha distillate in rats.

Article submitted for publication

(10) Mackay, D, A. DiGuardo, S. Paterson, & C. Cowan (1997)
EQC Model, ver. 1.01, 1997, available from the Environmental Modeling Centre, Trent University, Canada.

(11) Meylan, M, SRC 1994-1999.
LOGKOWWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(12) Meylan, M, SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(13) Schreiner, C. Bui, Q., Burnett, D., Koschier, F., Podhasky, P., Lapadula, E., White, R. and Schroeder, R. E. (1999)
Toxicity evaluation of petroleum blending streams: reproductive and developmental effects of light catalytic cracked naphtha distillate in rats.
J. Toxicol. and Env. Health, Part A, Vol 58, pp 365-382

(14) Springborn Laboratories, Inc. (1999)
Light Catalytically Cracked Naphtha-Determination of Inherent Biodegradability. Study No. 13687.6109

(15) Stonybrook Laboratories, Inc. (1995)
Static Renewal 96-hour acute toxicity of the water accommodated fraction (WAF) of FR 15799 FCC Light to Fathead Minnow. Study No. 66234
Stonybrook Laboratories Inc. Princeton, NJ.1995.

(16) Stonybrook Laboratories, Inc. (1995)
Static Renewal 48-hour Acute Toxicity of the Water Accommodated Fraction (WAF) of FR 15799 FCC Light (Light Catalytically Cracked Naphtha, LCCN) to Daphnia Magna. Study No. 66233.
Stonybrook Laboratories Inc. Princeton, NJ.1995.

(17) Stonybrook Laboratories, Inc. (1995)
Static Renewal 96-hour acute toxicity of the water accommodated fraction (WAF) FR 15799 FCC Light (Light Catalytically Cracked Naphtha, LCCN) to a Freshwater Alga, Selenastrum capricornutum, Study No. 66235
Stonybrook Laboratories, Inc Princeton, NJ.

(18) Stonybrook Laboratories, Inc.(1995)
Method Validation for the Analysis of Whole Light Catalytically Cracked Naphtha (LCCN) in Water Accommodated Fraction (WAF) using Purge-and-Trap and GC/FID,
Study No. 66232.
Stonybrook Laboratories, Inc. Princeton, NJ.1995

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # AROMATIC NAPHTHAS

Summary prepared by: American Petroleum Institute

Creation date: **1 MARCH 2001**

Printing date: **19 NOVEMBER 2001**

Date of last Update: **10 DECEMBER 2001**

Number of Pages: **37**

NB. Reliabiliy of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : petroleum product
Physical status : liquid
Remark : Aromatic naphtha streams are obtained from the catalytic reforming of mainly n-alkane and cycloparaffinic feedstocks into aromatic and branched chain hydrocarbons. They are mainly in the range C_5 to C_{12} and boil in the range of approximately 35 to 230°C.

Several materials have been employed in tests for toxicological properties of this category of products.

Details of the various samples are as follows:

API 83-05 is a sample of a catalytically reformed naphtha.
LCRN is a sample of Light Catalytically Reformed Naphtha
LCRN-D is a 154 °F distillation fraction derived from LCRN.
Characterization of these samples is shown in the following table.

Parameter	Sample		
	API 83-05	LCRN	LCRN-D
Gravity (°API)	44		
Sulfur (wt %)	<0.01		
Nitrogen (ppm)	<2		
RVP (psia)	3		
IBP (°F)	132		
FBP (°F)	389		
Olefins (% by MS)	1.5	0.96	1.37
Paraffins (% by MS)	31	63.76	88.3
Naphthenes (% by MS)	5	2.28	1.24
Aromatics (% by MS)	62.5	33	9.09
Benzene (vol %)	2.2	6.93	4.65
Carbon No. (vol%)			
4		1.02	3.6
5		23.34	59.11
6		25.76	25.18
7		45.27	11.65
8		4.56	0.46
9		0.05	0

19.11.2001

2.1 MELTING POINT

Not relevant

2.2 BOILING POINT

35 to 230 ºC see General substance information

2.5 PARTITION COEFFICIENT

Log pow	:	2.13 - 4.5 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65.
Year	:	2000
GLP	:	No
Test substance	:	Light Catalytically Reformed Naphtha
Remark	:	Log P values represent the spread of calculated and/or measured values for C_5 to C_9 hydrocarbon components found in LCRN, CAS No 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability	:	(2) valid with restrictions

26.06.2001 (19)

Log pow	:	2.13 - 4.76 at 25° C
Method		Calculated by LOGKOWWIN ver. 1.65.
Year	:	2000
GLP	:	No
Test substance	:	Full -Range Catalytically Reformed Naphtha (FRCRN)- CAS No. 68955-35-1; API sample 83-05
Remark	:	Log P values represent the spread of calculated and/or measured values for C_5 to C_9 hydrocarbon components found in FRCRN, CAS No 68955-35-1. Detailed hydrocarbon analysis was used to identify the components of this FRCRN (63% aromatics) sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.
Reliability	:	(2) valid with restrictions

11.11.2001 (19)

2.6.1 WATER SOLUBILITY

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	Yes
Test substance	:	Light Catalytically Reformed Naphtha
Method	:	Water Accommodated Fractions (WAFs) of LCRN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of selected components freshwater and saltwater solubilities of 13.7 and 14.0 ppm respectively. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Concentrations for these components reached equilibrium by 24 hours.
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LCRN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.
Reliability	:	(2) valid with restrictions

19.11.2001 (1) (11) (13) (15) (16)

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	yes
Test substance	:	Full -Range Catalytically Reformed Naphtha (FRCRN)- CAS No. 68955-35-1; API sample 83-05
Method	:	Water Accommodated Fractions (WAFs) of CONCAWE Reformate light naphtha (LCRN), CAS no. 64741-63-5 (CONCAWE sample ID W94/812) were prepared at 100 mg/l loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Remark	:	Detailed hydrocarbon analysis was used to identify the components of this CONCAWE Light Cracked Naphtha (63% aromatics) sample. The analysis indicated that the composition of the CONCAWE LCRN sample was essentially identical to the composition of API 83-05 FRCRN sample. Therefore the water solubility information for the CONCAWE LCRN sample is applicable to the FRCRN sample.
Result	:	Gas chromatographic analysis of LCRN components benzene, toluene, ethylbenzene, ortho, meta and para-xylene in WAFs indicated freshwater solubility of 6.3 ppm. Concentrations for these components reached equilibrium by 48 hours.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for FRCRN components range from approximately 3 to 2000 mg/l. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.

Reliability : (2) valid with restrictions
19.11.2001 (11) (12) (13) (15) (16)

3.1.1 PHOTODEGRADATION

Type	:	calculated
Light source	:	Sun light
Rel. intensity	:	= 1 based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	no
Test substance	:	Light Catalytically Reformed Naphtha
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_8 hydrocarbon components found in LCRN, CAS No. 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LCRN constituents is: 1.498 days (2,3 dimethyl pentane) to 15.985 days (isopentane).
Result	:	Indirect photolysis: Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate constant: 0.6691E-12 cm^3/mol-sec (isopentane) to 7.1392E-12 (2,3 dimethyl pentane) Half life: 1.498 to 15.985 days
Reliability	:	(2) valid with restrictions

26.06.2001 (20)

Type	:	Calculated
Rel. intensity	:	= 1 based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Method	:	Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	:	2000
GLP	:	no
Test substance	:	Full -Range Catalytically Reformed Naphtha (FRCRN) CAS No. 68955-35-1; API sample 83-05
Remark	:	AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O_3. Atmospheric oxidation rates were calculated for the C_5 to C_9 hydrocarbon components found in FRCRN, CAS No. 68955-35-1. Detailed hydrocarbon analysis was used to identify the components of this specific FRCRN (63% aromatics) sample. Based on a 12-hour day, the range for atmospheric half-lives for FRCRN constituents is: 0.641days (1, 2, 4 trimethylbenzene) to 15.985 days (isopentane).
Result	:	Indirect photolysis: Conc. of sensitizer: 1.50E+06 OH radicals/cm^3 Rate constant: 0.6691E-12 cm^3/mol-sec (isopentane) to 16.698E-12 (1,2,4 trimethyl benzene) Half life: 0.641 to 15.985 days
Reliability	:	(2) valid with restrictions

19.11.2001 (20)

3.1.2 STABILITY IN WATER

Test substance : Light Catalytically Reformed Naphtha
Conclusion : Hydrolysis unlikely
Reliability : (1) valid without restriction
26.06.2001 (17)

Test substance : Full -Range Catalytically Reformed Naphtha (FRCRN)
CAS No. 68955-35-1; API sample 83-05
Conclusion : Hydrolysis unlikely
Reliability : (1) valid without restriction
11.11.2001 (17)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment and sediment
Year : 2000
Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_8 hydrocarbon components found in LCRN, CAS No 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample.

The majority of LCRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

Medium	% distribution
Air	97 to 99.98
Soil	0.01 to 0.8
Water	0.01 to 2.7
Sediment	0.00
Suspended sediment	0.00

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.
Reliability : (2) valid with restrictions
05.10.2001 (18)

Type : Calculated according to Mackay Level I
Media : Soil, air, water, suspended sediment, and sediment
Year : 2000
Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C_5 to C_9 hydrocarbon components found in FRCRN, CAS No 68955-35-1. Detailed hydrocarbon analysis was used to identify the components of this specific FRCRN (63% aromatics) sample.
The majority of FRCRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result : Medium — % distribution

Medium	% distribution
Air	96.5 to 99.98
Soil	0.01 to 1.83
Water	0.01 to 2.7
Sediment	<0.001 to 0.03
Suspended Sediment	<0.001

Test substance : Full -Range Catalytically Reformed Naphtha (FRCRN)
CAS No. 68955-35-1; API sample 83-05

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.

Reliability : (2) valid with restrictions
11.11.2001 (18)

3.5 BIODEGRADATION

Type : aerobic
Inoculum : mixed, adapted inoculum of domestic activated sludge and soil
Contact time : 56 day
Result : inherently biodegradable
Year : 1999
GLP : yes
Test substance : Light Catalytically Reformed Naphtha
Method : CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593

: Type (test type): Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)

Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO_2 production. By day 28 approximately 96% of the test material was degraded, then essentially reached a plateau in degradation rate until day 56. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63% actual) was observed by day 14, and total blank CO_2 production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21 ºC, which deviated from the protocol value of 22 ±2º. This deviation was not expected to have affected the outcome of this study.

	% Degradation (sd)	
Test Day	Hexadecane	Test Material
3	13.93 (1.85)	30.85 (3.85)
7	34.40 (4.54)	53.71 (3.52)
14	63.17 (0.94)	77.25 (3.65)
21	77.26 (6.52)	87.17 (8.87)
28	90.35 (7.14)	96.17(5.26)
35	85.13 (n=1)	107.9 (n=1)
42	85.21 (n=1)	96.95 (6.37)
49	96.93 (8.94)	92.02 (n=1)
56	94.69 (4.10)	84.92 (0.51)

Test condition : Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two-week adaptation period. Triplicate test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation

procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass-distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of soil was obtained at a depth between 5-10cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substances were added directly to test systems using a 10 microliter Hamilton gas tight syringe. The volume required to achieve the specified mg carbon/l concentrations were calculated based on % carbon and specific gravity of the respective substance. The test substance % carbon (0.8856) and specific gravity (0.7325 mg/µl) information was supplied by the Sponsor. Hexadecane % carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/µl) was obtained from Verschueren (1983). Addition of respective substance was performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 º (± 2ºC) in the dark.

Biodegradation by CO_2 determination
Test initiation and procedure
On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume(100 ml inoculum/l).Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/l concentration. Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO_2 analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22 º(± 2 º C).
On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc H_3PO_4 was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, then analyzed for CO_2 using gas chromatography-thermal conductivity detection. Quantitation

of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability : (1) valid without restriction
19.11.2001 (24)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	96 Hr Static Acute Toxicity Test w/ Daily Renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	Mg/l
Analytical monitoring	:	yes
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year	:	1998
GLP	:	yes
Test substance	:	Light Catalytically Reformed Naphtha
Method	:	Statistical Method: (FT - ME) LL_{50} and LC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 1, 0, 1, 0, 1 and 20 in 0, 3.1, 6.3, 13, 25 and 50 mg/l treatments. Abnormal behavior (surfacing, erratic swimming) was observed at 96 hrs for 3 organisms in 13 mg/l and 7 fish in 25 mg/l treatments. 96-hr LL_{50} = 34 mg/l, 25-50 mg/l w/ 95% C.I. (as nominal loading rate) 96-hr LC_{50} = 11 mg/l, 8.2-17.2 mg/l mg/l w/ 95% C.I. (measured concentrations) 96-hr NOEL =3.1 mg/l (nominal); 96-hr NOEC =1.03 mg/l (measured) based on lack of mortality and abnormal effects for these treatments.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by blending naturally hard well water with water that had been demineralized by reverse osmosis. Nominal loading rates of 0, 3.1, 6.3, 13, 25 and 50 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. After stirring for 24 hrs using 25%or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes.

Fish were hatched and raised from ABC Laboratories' in-house culture, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8-liter glass containers with teflon-lined caps. Fish were acclimated to the test water and temperature approximately 72 hr before the test, and were not fed during this 72 hr period. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving approximately one liter of solution to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Water temperature was 21-22 ºC. Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 6.0 and 8.5, pH values between 7.7 and 8.5. Hardness values ranged from 138 to 148 mg/l; alkalinity values ranged from 150 to 158 mg/l and conductivity values ranged from 299 to 313 microsiemens.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

19.11.2001 (2)

Type : Predicted value for fish toxicity
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : 2.09
Method : Predicted
Method : Calculated based on hydrocarbon block principle. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below).
Result : Estimated 96 hour(s) fish acute toxicity LL_{50}: 2.09 mg/l
Reliability : (2) valid with restrictions
11.11.2001 (21)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	Mg/l
Analytical monitoring	:	Yes
Method	:	Procedure patterned after:1991 ASTM method E729-88a and 1985 USEPA TSCA Test Guidelines: Daphnid Acute Toxicity Test. Fed. Reg., vol. 50 (No. 188) Sept 27, 1985, 797.1300. Statistical Method: (FT - ME) EL 50 and EC50 calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Year	:	1998
GLP	:	Yes
Test substance	:	Light Catalytically Reformed Naphtha
Result	:	Immobility (no. of organisms) at 48 hrs: 0, 0, 0, 15, 20 and 20 for 0, 3.0, 6.0, 12, 24 and 48 mg/l treatments. At the 6 and 12mg/l nominal treatments, 20 and 5 organisms were observed at the bottom of the test chambers, respectively. 48-hr EL_{50} = 10 mg/l based upon nominal loading rate (95% C.I. 6 to 12 mg/l); 48 hr EC_{50} was 2.6 mg/l (95% C.I. 1.06 to 3.6 mg/l); based on total measured concentrations. 48-hr NOEL = 3 mg/l based upon nominal loading rate. 48 hr NOEC was 0.465 ppm based on total measured concentrations.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were a blend of aged well water and reverse osmosis well water. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 2.4 liters of water for 24 hr in aluminum foil covered 2.5 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately one hour, then drained from the port and used for testing. Samples were also analyzed by purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes. Range finding toxicity studies were conducted at 0.5, 1.0, 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 3.0, 6.0, 12, 24 and 48 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were teflon cap-sealed 273 ml glass jars with 10 daphnids per jar and were completely filled with test

solution.

During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.5; pH ranged from 8.2 to 8.4; temperature was 20 to 21 ºC; hardness (mg/l) ranged from 146 - 152; alkalinity (mg/l) was 158-168 and conductivity (umhos) values were 312 - 317.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1998.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components; remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

19.11.2001 (3)

Type : Predicted value for invertebrate 48 hour acute toxicity
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 0.9
Method : Calculated based on hydrocarbon block principle. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below).
Result : Estimated 48 hour(s) Daphnid acute toxicity EL_{50}: 0.9 mg/l.
Reliability : (2) valid with restrictions
19.11.2001 (21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Analytical monitoring : yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1998
GLP : yes
Test substance : Light Catalytically Reformed Naphtha
Method : Statistical Method: EL 50 and EC50 calculated using nonlinear logistics sigmoid model (SAS). All NOEL/NOEC values based on visual review and Dunnett's test for significance.
Result : Percent inhibition on growth determined by cell density (cells/ml):
96 hour EL_{10}=6.0 mg/l (3.1-8.8 mg/l CI @95%)
96 hour EL_{50}=8.5mg/l (7.3-9.8 mg/l CI @95%)
96 hour EL_{90}=12 mg/l (9.9-14 mg/l CI @95%)
96 hour NOEL=5.0 mg/l

96 hour EC_{10}=1.1 mg/l (0.41-1.8 mg/l CI @95%)
96 hour EC_{50}= 1.7mg/l (1.4-2.1 mg/l CI @95%)
96 hour EC_{90}=2.7 mg/l (2.1-3.4 mg/l CI @95%)
96 hour NOEC=0.866 mg/l

Subcultures of the 10, 20 and 40 mg/l treatment cultures were placed in fresh media (no test substance) after acute

testing for ten days indicated that growth inhibition was algistatic in all treatments. No excursions from the protocol were noted which would have affected the integrity of the study.

Concentration (mg/l)

Nominal	Measured	96hr cell density (cells/ml)
Control	0.0147	40.5 $x10^4$
1.3	0.126	40.92 $x10^4$
2.5	0.211	42.33 $x10^4$
5.0	0.866	41.17 $x10^4$
10	2.12	11.11 $x10^4$
20	5.26	0.70 $x10^4$
40	13.3	0.04 $x10^4$

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.4 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 µg/l EDTA chelator, pH adjusted to 7.5 + 0.1 with 0.1NHCl and sterilized by 0.45 micron filtration) in 2.5 liter aspirator bottles. The mixing vessels were sealed with foil covered stoppers, covered with aluminum foil and the contents mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed from the spout at the base of each bottle and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (148 ml) with treatment solution and inoculated with 6 day old algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media. Original algal cultures (stock UTEX-1648) obtained from Dept of Botany, Culture Collection of Algae, University of Texas at Austin, 1997. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 1.3, 2.5, 5.0, 10, 20 and 40 mg/l. The initial algal concentration was 1.0 x 103 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination range 371 to 442 ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0,24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes.

Test temperature was 24-25 ºC. Test solution pH ranged from 7.5 to 8.9.

Reliability : (2) valid with restrictions
Measured concentrations may not represent 100% of components; remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

19.11.2001 (4)

5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle : undiluted
Year : 1985
GLP : yes
Test substance : API 83-05
Method : The test material was administered undiluted, as a single oral dose to groups of 5 male 5 female rats at dose levels ranging from 3.57 to 9.8 g/kg. The dose volume varied per dosage level based on an average bulk density of 0.8 g/ml. Food had been withheld from the rats overnight prior to dosing, but they had free access to water. Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days.
The rats were weighed the day before dosing and then at 7 and 14 days after dosing.
All animals, whether dying during the study or surviving to termination were subjected to a gross necropsy and any abnormalities were recorded.

Result : Clinical signs seen during the study included: hypoactivity, diarrhea, brown-stained anal area, ataxia, prostration, red stained nose and mouth, lacrimation, dyspnea, yellow-stained abdomen, hair loss on abdomen, decreased limb tone, piloerection, hair loss on front legs, excess salivation, yellow- or reddish-brown-stained urogenital region, tremors and death.
All mortality occurred within the first three days after dosing. All surviving animal had returned to normal by day 11 except for those with hair loss.

At necropsy, there were few findings in the animals that survived to termination.
Mortalities and body weights were as follows:

Dose level (g/kg)	Body weights (g)		Mortality	No with lesions at necropsy
	Initial (fasted)	Terminal		
Male				
5	285	336	0/5	0/5
6	315	370	1/5	1/5
6.5	324	386	1/5	1/5
7	306	-	5/5	4/5
9.8	310	-	5/5	5/5
Female				
3.57	212	227	0/5	1/5
4.29	199	233	0/5	0/5
5	202	235	3/5	3/5
7	210	213	3/5	2/5
9.8	208	-	5/5	5/5

Typically at non-lethal dose levels the lesions frequently observed included: presence of dark colored material in the stomach, glandular mucosa of stomach with dark red to black

area. Additionally at the highest dose levels the urinary bladder contained a red fluid in 4 of the five males examined.

The estimated LD_{50} values and 95% confidence limits were:
Males: 6.62 g/kg (6.2 - 7.08)
Females: 5.39 g/kg (3.23 - 6.86)

Reliability : (1) valid without restriction
19.11.2001 (6)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	5
Vehicle	:	air
Exposure time	:	4 hour(s)
Value	:	> 5.22 mg/l
Year	:	1984
GLP	:	yes
Test substance	:	API 83-05
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-05 at a nominal concentration of 5mg/l for 4 hours. After the 4-hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.
Result	:	The exposure chamber TWA concentration was determined to be 5.22±0.14 mg/l. No animal died during the study and no clinical signs of systemic toxicity were observed. There were no significant gross observations at necropsy and no histological changes were observed in the lungs. The 4 hour LC_{50} was therefore greater than 5.22 mg/l.
Reliability	:	(1) valid without restriction

19.11.2001 (5)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Species	:	Rat
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	4
Vehicle	:	undiluted
Value	:	> 2000 mg/kg bw
Year	:	1985
GLP	:	yes
Test substance	:	API 83-05
Method	:	The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to

a dose of 2 g/Kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. Collars were used to restrain the animals during the application period. The animals were observed for a total of 14 days post-dosing.
Body weights were recorded just prior to dosing and again seven and 14 days after dosing.
At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.

Result : There were no mortalities during the study and there were no clinical signs of toxicity with the exception of one rabbit. This animal had soft stools, diarrhea, hypoactivity and an inflamed urogenital area during the last three days of the study.
All animals had gained weight by the end of the study.
Dermal irritation occurred during the study and this ranged from slight to severe for erythema, slight to marked for edema and slight to marked for atonia, desquamation, coriaceousness and fissuring.
At necropsy, the only findings in the males were on the treated area of the skin and were consistent with the gross observations of irritation. In the females similar skin lesions were observed and in addition, the vagina was reddened in 3 of the four animals and in one of these the trachea contained a red liquid on the inside walls and the lungs had multiple red pinpoint foci on all lobes.

Reliability : (1) valid without restriction
19.11.2001 (6)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Not relevant

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : 3.1
Method : Draize Test
Year : 1985
GLP : yes
Test substance : API 83-05
Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing.
After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 5, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : The scores for erythema and edema at each of the observation times were as follows:

	Erythema		Edema	
	Intact	Abraded	Intact	Abraded
24 h	1.2	1.5	1.5	1.8
72 h	1.5	1.5	1.7	1.8
5 days	1.0	1.3	1.5	1.7
7 days	0.8	1.0	1.0	1.0
14 days	0	0	0	0

The Primary dermal Irritation index was 3.1

Growth rates were normal throughout the study and there were no clinical signs of systemic toxicity.

Reliability : (1) valid without restriction
02.03.2001 (6)

5.2.2 EYE IRRITATION

Species : Rat
Concentration : Undiluted
Dose : 0.1 ml
Comment : rinsed eye after 30 sec exposure in 3 rabbits only
Number of animals : 9
Method : Draize Test
Year : 1985
GLP : yes
Test substance : TS: API 83-05
Method : 0.1 ml of undiluted test material was dripped onto the corneal surface of one eye of each of 9 rabbits; the other eye was untreated and served as control.
After 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed.
Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. At the 72 hour and seven day readings, sodium fluorescein was used to aid in revealing possible corneal injury.
Body weights were recorded just prior to treatment and one week afterwards. At termination of the study, the rabbits were euthanized by an overexposure of carbon dioxide and were subjected to a gross necropsy. Any abnormalities found were recorded.

Result : No signs of systemic toxicity were observed during the study.
The primary eye irritation scores were as follows:

Observation period	Primary eye irritation score	
	Unwashed*	Washed**
1 hour	7.2	7.3
24 hour	5.5	2.7
48 hour	4.3	2.0
72 hour	3.0	2.0
7 day	1.0	1.3
14 day	0	0

* Mean of six rabbits
** Mean of three rabbits

In rabbits whose eyes had not been washed, irridial irritation that had occurred had subsided by 24 hours and

all corneal involvement had subsided by 48 hours.
No corneal or irridial irritation was observed in the group whose eyes had been washed followed by application of test material.

Reliability : (1) valid without restriction
19.11.2001 (6)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	guinea pig
Concentration	:	Induction 50 % occlusive epicutaneous Challenge 25 % occlusive epicutaneous
Number of animals	:	20
Vehicle	:	Paraffin oil
Result	:	not sensitizing
Year	:	1986
GLP	:	yes
Test substance	:	API 83-05
Method	:	0.4 ml of a 50% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.4 ml of a 25% mixture in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose, the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream. Positive control (2,4-dinitrochlorobenzene), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups. The positive control was used at a concentration of 0.3% in 80% aqueous ethanol for the induction doses and at 0.1% w/v in acetone for the challenge dose.
Result	:	There was no abnormal appearance in any of the animals exposed to the test material during the study. The skin reactions to the challenge dose are summarized as follows: Test material: No dermal reactions by any animal Naive control: Very slight erythema in 2/20 animals Vehicle control: No dermal reactions by any animal Positive control: Very slight to moderate irritation by all 20 animals. The reactions of 16 of the animals exceeded the highest reaction observed in the naive positive control animals. Naive positive control: 10/20 animals exhibited very slight erythema.
Reliability	:	(1) valid without restriction

19.11.2001 (10)

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	13 weeks
Frequency of treatment	:	6 hours/day, 5 days/week
Post obs. period	:	Recovery group 4 weeks
Doses	:	750, 2500 and 7500 ppm
Control group	:	yes
NOAEL	:	2500 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	2000
GLP	:	yes
Test substance	:	LCRN-D (distillate of LCRN)
Method	:	The method used was described in OECD guideline 413. Groups of 16 male and 16 female rats underwent whole body exposures to 750, 2500 and 7500 ppm LCRN-D. Exposures were for 6 hours each day, 5 days per week for 13 weeks. Extra groups of 16 rats of each sex were exposed to the high dose level and also for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 13 weeks exposure. Neurobehavioral evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14 and 18 9ecovery groups. Animals were not exposed to LCRN-D during these tests. Following 13 weeks of exposure, 16 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically. At the end of the 4 week recovery period, 16 animals of each sex from the high and control groups were necropsied and selected tissues were examined microscopically. During the study, clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and 19 weeks (recovery groups). Body weights and food consumption was measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements. At termination (after 13 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. 10 animals of each sex were designated for non-neuropathological examination and 6 of each sex for neuropathological examination. For the non-neuropathology animals, the following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) was removed from the control and high dose animals, were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination.
Animals designated for neuropathological examination were subjected to a detailed examination of central and peripheral nervous tissues. N

Neurobehavioral studies were undertaken as follows:

Motor activity
Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 14 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery
An assessment of the following was made:

Home cage evaluations for Posture, vocalization, palpebral closure.

Handling evaluations for reactivity to general stimuli, signs of autonomic function.

open field behavior: arousal level, gait, urination and defecation frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.

Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result **:** There were no mortalities during the study and there were no treatment-related signs of toxicity. The ophthalmic examinations did not reveal any treatment-related effects. Mean body weights, body weight gains and food consumption were unaffected by treatment.

No treatment-related effects were recorded in the Functional Operational Battery. In the examinations of motor activity, there were no treatment-related effects recorded during the 13 week exposure period but a slight increased activity was found in the highest treatment group after the 4 week recovery period.

After 13 weeks exposure there was a significant decrease in total WBC count (36%) and lymphocyte counts in the high dose males and a slight decrease in neutrophil counts for the mid dose males. A trend towards decreased WBC (2.1%) and lymphocyte counts was also seen in the mid dose males and high dose females. After the 4 week recovery period, leukocyte values were comparable to control values. However, MCV was slightly decreased (2.8%) in the high dose males. It was concluded that these changes were suggestive of a reversible slight effect of the LCRN-D.

Clinical chemistry parameters were unaffected by treatment.

After 13 weeks exposure relative kidney weights in the high dose males were increased (15.9%) and this correlated with the occurrence of hyaline droplets in the proximal convoluted tubules. This finding has been described as a "light hydrocarbon nephropathy" and is sex and species specific and is not relevant for human health risk assessment.
In the high dose males decreased absolute (25.7%) and relative (22%) spleen weights were also recorded. It was concluded that this was associated with the minor hematological changes that had been observed. These differences were not apparent after the recovery period and no abnormal microscopic findings were found in either the spleen or bone marrow.

Brain length and width measurements were unaffected by treatment and there were no abnormal microscopic findings in the brain, spinal cord or peripheral nerves.

Reliability : (1) valid without restriction
19.11.2001 (22)

Species	:	Rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	13 weeks
Frequency of treatment	:	6 hours/day, 5 days/week
Doses	:	410, 1970 and 8050 ppm
Control group	:	Yes
NOAEL	:	1970 ppm
Year	:	1996
GLP	:	no data
Test substance	:	Partially vaporized full range catalytic reformed naphtha
Method	:	Groups of 15 rats of each sex were individually housed in $1m^3$ inhalation chambers. The rats underwent whole body exposures to partially vaporized full range catalytic reformed naphtha (FRCRN). Exposures were [6 hours/day, 5 days/week] for 13 weeks at nominal concentrations of 500, 2000 and 8000 mg/m^3. Two extra groups of 15 rats of each sex served as sham and untreated controls. (NB. This is not stated in the publication but from other comments in the paper, it is clear that exposure was not continual during the study). Water was available ad lib, but food was withheld during the exposure periods. Clinical observations were made regularly and body weights were recorded weekly. At the end of the 13 weeks exposure, blood samples were taken for hematological and clinical chemical measurements. The rats were then sacrificed and necropsied. Organs were weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet weight of the right middle lung lobe was also weighed. The lobes were then dried and their dry weights determined. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to determine the number of testicular spermatids.

The following tissues from the high dose animals were examined histologically: adrenals, bone and marrow (sternum), pancreas (head), brain (three sections), submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach (squamous and glandular), colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver, tracheobronchial lymph nodes, lung (left lobe), nasal turbinates (four sections), thigh muscle, urinary bladder, sciatic nerve, and any gross lesions. In addition, tracheobronchial lymph nodes and any gross lesions from untreated control animals were also evaluated.

Result : There were no treatment-related clinical signs during the study, no effects on serum chemistry values or parameters of the male reproductive system a terminal sacrifice.
Body weights of males were exposed t the mid and high dose groups were higher than the controls throughout the study and the differences were statistically significant in the high dose group from week 10 onwards.

WBC count was significantly lower in sham treated controls and all three treated groups in both sexes compared to untreated controls. Additionally the WBC count was decreased by approximately 24% in the high dose females when compared to the sham controls. No other parameters were affected.

The only organ weights affected were the liver and kidney. In the male high dose group, mean kidney weight was approximately 13% greater than the sham treated animals (but not the untreated controls), and the liver weight was approximately 14% greater.
No treatment-related gross lesions were observed at necropsy and no treatment-related abnormalities were noted during microscopic examination. Because of the lack of effects in the histology, no tissues were examined from the lower dose groups.

Test substance : Test atmospheres were generated by partially vaporizing FRCRN. The concentrations in the chamber were adjusted by dilution with air. Concentrations were monitored throughout the study The actual concentrations for each of the dose levels are shown below.

	Exposure group		
Parameter	Low	Medium	High
Target conc. (mg/m3)	500	2000	8000
Actual conc. (mg/m3)	410	1970	8050
Butane	4.33	3.91	4.05
Methylbutane	20.56	17.26	17.55
Pentane	13.24	11.44	11.86
Hexane	6.53	5.71	6.36
Heptane	2.32	2.35	2.33
Benzene	2.19	4.93	5.79
Toluene	10.02	12.23	10.93
m- and p- Xylenes	3.57	4.05	3.4
2- Ethyltoluene	0.43	0.35	0.17
Trimethylbenzene	0.01	0.01	0.04

Reliability : (2) valid with restrictions
The publication is not clear in its description of the frequency and duration of exposures. However, it is assumed that the exposures are 6 hours/day, 5 days/week since this would be consistent with other studies reported from the same laboratory.

19.11.2001 (14)

Species	:	rabbit
Sex	:	male/female
Strain	:	New Zealand white
Route of admin.	:	dermal
Exposure period	:	6 hours
Frequency of treatment	:	3 times per week for 28 days (12 applications total)
Doses	:	200, 1000 & 2000 mg/kg
Control group	:	yes, concurrent no treatment
Year	:	1986
GLP	:	yes
Test substance	:	API 83-05
Method	:	Prior to the study a 5-day range finding study was conducted. The method and results of the range-finding study are not included in this summary. Undiluted API 83-05 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours, which was then removed and the skin was wiped with a dry gauze to remove any residual material. A group of five rabbits of each sex served as sham controls. The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly. At termination, blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination. A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination.
Result	:	Two males in the 2000 mg/kg group and one male in the 1000 mg/kg died during the study. The deaths occurred on days 12 and 17 for the highest dose group and day 19 at the mid dose group. There were no clinical signs of intoxication in any other animal on the study. At 200 and 1000 mg/kg there were no treatment related effects on body weight gains over the study period although there were isolated differences between treated and control animals during the study. At the highest dose level, the females showed no weight gain and the males had an overall weight loss. A mean irritation score was calculated for each day and overall means were also calculated. (The mean irritation score, MIS, was the sum of irritation scores for both erythema and edema for all animals of a given dose group and sex.) The overall MIS for each dose group was:

Group (mg/kg)/sex	MIS	Classification
2000 M	5.1	Severe irritant
2000 F	4.9	Severe irritant*
1000 M	4.3	Moderate irritant
1000 F	4.1	Moderate irritant
200 M	2.9	Moderate irritant
200 F	2.5	Moderate irritant
Control M	0	Non irritant
Control F	0	Non irritant

*Severe irritation was observed in the high dose females during the study and the authors concluded that even though the overall MIS for this group led to a moderate classification, a severe classification would be more appropriate.
Some differences were observed between the control and treated groups for a few hematological and clinical chemical parameters. The differences from control values are shown below. However, the authors concluded that since the values fell within the normal range for the laboratory, they should not be regarded as treatment related.

Dose group	Parameter	Difference
1000 mg (M)	WBC	25% higher
	Alkaline phosphatase	37% lower
2000 mg (M)	Hemoglobin	5% lower
	Blood urea nitrogen	24% lower
	Alkaline phosphatase	35% lower
2000 mg (F)	SGPT	26% lower

There were also a few differences between control and treated animals for absolute and relative organ weight for a small number of organs. Since there was no associated histopathological findings and since the differences were not dose-related, they were not considered to be significant.
At necropsy, few gross findings were recorded other than effects on the treated skin. The findings in the liver of males and females of treated and control groups consisted of areas of discoloration and were considered to incidental to treatment.
Histological changes were mainly confined to the skin except for two males that died in the highest dose group. The kidneys of these two animals contained slight to moderate tubular degeneration.
The changes in the skin consisted of slight to moderate proliferative and inflammatory changes at the highest dose group. Concurrent with these changes in the skin there was an increased granulopoiesis of the bone marrow, probably related to the stress or other factors associated with the skin irritation. Such changes were not observed in the control animals.

Reliability **:** (1) valid without restriction
19.11.2001 (9)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Mouse lymphoma assay
System of testing	:	Forward mutation assay using cell line L5178Y TK+/-
Cycotoxic conc.	:	250µl/ml, lethal at 500µl/ml
Metabolic activation	:	with and without
Year	:	1985
GLP	:	yes
Test substance	:	API 83-05
Method	:	The test material was dissolved in acetone for this assay. Three positive control substances were used viz Ethyl methane sulphonate (EMS) at concentrations of 0.25 &0.4 µl/ml for the non activation assay, Dimethylnitrosamine (DMN) at a concentration of 0.3 and Methylcholanthrene (MCA) at a concentration of 2.5 µg/ml for the activation assay. A cytotoxicity study was carried out prior to the mutagenicity assay. The test material was lethal at a concentration of 500 µl/ml and highly toxic at 250 µl/ml without S-9. These results were used to select a dose range of 6.25 to 500 µl/ml for the non-activation assay and 3.13 to 400 µl/ml for the activation assay. For the mutation assay, the lymphoma cells were exposed for 4 hours to test material. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; TFT was used as the restrictive agent. six non-activated and six activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 6.25, 25, 37.5 50, 75 and 100µl/ml of test material and resulted in a range of growth of 30 to 97% compared to the solvent control. The activated cultures that were cloned were treated with 18.8, 37.5, 75, 100, 150 and 200 µl/ml of test material. This resulted in growth ranging from 4.6 to 67.9% compared to solvent control. Plates were prepared from TFT and from the VC cultures and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was then determined. The following criteria were used in judging the significance of the activity of the test article. Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level. Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background. Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.
Result	:	The mutant frequencies and the percentage total growth at each of the test concentrations is summarized in the following table.

Concentration (µl/ml)	Mutant frequency	% Relative growth
Non-Activated		
6.25	24.2	97.3
25	22.5	64.3
37.5	18.2	32.6
50	23	47.8
75	39.6	59.4
100	22.3	29.6
Solvent 1	100	22.7
Solvent 2	100	30.6
Untreated control	20.7	110.6
EMS 0.25 µl/ml	364.5	53.8
EMS 0.4 µl/ml	504.5	23.2
S-9 Activated		
18.8	54.2	67.9
37.5	57.3	56.1
75	72.1	60.3
100	85.2	32.8
150	73	27.4
200	146.2	4.6
Solvent 1	31.3	100
Solvent 2	30.8	100
Untreated control	42.1	123.9
DMN 0.3 µl/ml	258.8	12.7
MCA 2.5 µl/ml	243.6	78.5

The authors concluded that the test material was not mutagenic in the non-activated assay because there was no dose response relationship and furthermore the mutant frequency was not significantly different from the solvent and untreated controls.
The minimum criteria for indicating mutagenesis would have been $47x10^{-6}$. Since the 100 µl/ml treatment represented a close approach to the excessively toxic treatment at 150 nl/ml, this assay was considered sufficient to evaluate the test material as non-mutagenic under non-activation conditions.

In the presence of the S-9 mix, the test material was converted into one or more mutagenic products.
The minimum criterion for a significant response was a mutant frequency exceeding $62.1x10^{-6}$. This value was exceeded for 4 of the 6 analyzed cultures. The response was dose related.
The results were judged sufficient to evaluate the test material as mutagenic in the presence the metabolic activation system.

Reliability : (1) valid without restriction
19.11.2001 (8)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : one single dose given
Doses : 0.26, 0.82 & 2.42 g/kg
Result : negative
Year : 1985
GLP : yes
Test substance : API 83-05
Method : Two studies were carried out. In the first study, the test material did not induce a significant increase in the percentage of aberrant cells above the controls in either sex. Furthermore, the positive control (TEM at a dose of 0.8 mg/kg)) did not induce a significant elevation in the percentage of cells with structural aberrations. The assay was, therefore, repeated using a higher dose of TEM.
In this summary, only the results of the repeat study are described.

The study design was as follows:

Treatment	Animals/sex/sacrifice time		
	6 hrs.	24 hrs.	48 hrs
Corn oil (vehicle)	5	5	5
API 83-05, 2.42 g/kg	5	5	5
API 83-05, 0.82 g/kg	5	5	5
API 83-05, 0.26 g/kg	5	5	5
Triethylenemelamine (Positive control)		5	

Test material in vehicle was given intraperitoneally at a dose of 5 ml/kg to groups of rats as shown above. Corn oil was used as vehicle control and TEM (1.5 mg/kg) as the positive control.
Four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (4 mg/kg).
One male in the 2.42 g/kg group and one male in the 0.82 g/kg dose group died immediately after dosing, these were replaced by substitute animals.

Immediately after sacrifice, bone marrow was obtained from the tibiae of the animals. The marrow was washed and the cells were fixed before being spread on slides (at least 3 from each animal) for examination.
Slides were scored for chromosomal aberrations.
Where possible, a minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy (1-3).
A mitotic index (= No. of cells in mitosis/500 counted X 100) was calculated and recorded.

The type of aberration, its frequency, the statistical significance of any increases and its correlation to dose in a given time period will all be considered in evaluating a test article as being mutagenically positive or negative.
Criteria for a positive response are generally a

statistically significant dose-related increase in the number of structural aberrations at three dose levels. The final decision is based on scientific judgment.

Result : The dose levels used in the assay were selected on the basis of a preliminary screen.
In the cytogenetics assay, one male died at each of the dose levels 2.42 and 0.82 g/kg, the mortality occurred immediately after dosing. Toxic signs included lethargy and a moribund appearance at the high dose and slow uncoordinated movement in the mid dose group.
The results of the cytogenetics evaluations are summarized in
the following table.

MALES

	Dose level (g/kg)			Positive	Vehicle
	0.26	0.82	2.42	control	control
Cells with 1 or more aberrations					
6 hrs	0.5	0.4	1.0		0.5
24 hrs	0.4	0.8	1.0	32.4	0
48 hrs	0	1.6	0.5		0.8
Cells with 2 or more aberrations					
6 hrs	0	0	0		0.5
24 hrs	0	0	0	10.8	0
48 hrs	0	0	0		0
Frequency of structural aberrations					
6 hrs	.005	.004	.01		.05
24 hrs .	004	.008	.01	.708	0
48 hrs	0	.016	.005		.008
Frequency of numerical aberrations					
6 hrs	.005	0	.016		.015
24 hrs	.008	.008	.01	.008	.005
48 hrs	.01	.008	0		.004
Mitotic Index					
6 hrs	4.1	3.6	2.4		5.4
24 hrs	4.4	5.7	5.5	6.3	5.0
48 hrs	6.5	5.1	5.2		5.7
FEMALES					
Cells with 1 or more aberrations					
6 hrs	0	0.5	1.6	0.8	
24 hrs	0	0.4	1.5	0	33.2
48 hrs	0	1.2	0.8	1.2	
Cells with 2 or more aberrations					
6 hrs	0	0	0	0	
24 hrs	0	0	0.5	0	13.2
48 hrs	0	0	0	0	
Frequency of structural aberrations					
6 hrs	0	.005	.016	.008	
24 hrs	0	.004	.02	0	.804
48 hrs	0	.012	.008	.012	
Frequency of numerical aberrations					
6 hrs	.005	.005	.020	0	
24 hrs	.01	.016	.005	.020	.020

48 hrs	.008	0	.012	.005	
Mitotic Index					
6 hrs	5.4	6.3	3.1	6.1	
24 hrs	4.9	5.4	4.1	4.8	4.7
48 hrs	5.5	4.9	7.0	5.2	

NB.1. For simplicity only, mean values without standard errors are shown in the above table although they are given in the laboratory report.

On the basis of the criteria defined for assessing the results, the authors concluded that API 83-05 was not mutagenic in this assay.

Reliability : (1) valid without restriction
19.11.2001 (7)

5.7 CARCINOGENITY

No data available

5.8 TOXICITY TO REPRODUCTION

Type : Reproductive/developmental toxicity screening study, (OECD 421)
Species : Rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Frequency of treatment : 6 hours/day, 7 days/week
Premating exposure period
Male : 2 weeks
Female : 2 weeks
Duration of test :
Doses : Target conc.: 750, 2500 & 7500 ppm. Actual conc.: 750, 2490 & 2490 ppm
Control group : yes
Method : OECD combined repeated dose and reproductive/developmental toxicity screening test
Year : 2000
GLP : yes
Test substance : LCRN distillate (LCRN-D)
Method : Groups of 10 rats of each sex were exposed to 750, 2500 or 7500 ppm. LCRN-D for 6 hours /day, seven days/week. A group of 10 rats of each sex served as sham treated controls. Parental females were exposed for 14 consecutive days prior to mating, throughout mating and days 0-10 of gestation. Dams and their litters were sacrificed on post partum day 4. Unmated females and parental males were exposed to the test material for 14 days prior to mating, throughout mating and 18 additional days following completion of the mating period. These animals were sacrificed shortly after the last litters were delivered reached post partum day 4.

Mating
Within each group one male was co-housed with the same female until evidence of mating was observed (presence of sperm in vaginal smear or copulatory plug). The day of mating was designated day 0 of gestation. Following mating, the females were housed individually and continued their

exposures to test material until day 19 of gestation.
Females not showing evidence of mating following a 14 day mating period continued their exposures. If such a female showed signs of being pregnant, it was removed from the exposure regimen and observed for parturition.

Observations
All parental animals were regularly observed for mortality and gross pharmacologic signs. A physical examination, including palpation for tissue masses was carried out daily 30 mins. after removal from the exposure chambers.
Body weights and food consumption were measured throughout the study.
From day 20 of gestation, females (pregnant and non-pregnant) were observed for signs of parturition.
As soon as possible after delivery, litters were observed for the number of live and dead pups and for any abnormalities. Litters were also observed twice daily for unusual findings and dead pups.
On days 0 and 4 of lactation, the pups wee counted, weighed and sex was determined by external observation.

Pathology
Males were killed as a group shortly after the last litters delivered had reached day 4 of lactation.
Females with litters that reached day 4 of lactation were killed the next day or shortly thereafter.
Unmated females and those that did not deliver were killed 23 days after completion of the mating period.
At post mortem, a complete macroscopic examination was carried out on all adult animals. The following organs were weighed and organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, lung, spleen, epididymes, testes and thymus. Post mortem examination of females included a count of uterine implantation scars when present.

Pups were sacrificed on day 4 of lactation and underwent a complete macroscopic examination and a determination of sex by internal examination. All pups were preserved with viscera intact. Pups found dead at birth and that died prior to day 4 of lactation also underwent a gross external and internal examination. Dead pups were not eviscerated, the intact pups were preserved.

27 tissues were preserved from all adult animals in all dose groups. Ovaries, testes, epididymes, nose with nasal turbinates, and any grossly observed abnormalities were processed and sections examined histologically for all males and female parental animals in the control and highest dose group. Four sections were prepared and examined microscopically of the skull containing the nasal turbinates. These were

area between upper incisor and incisive papilla
area between incisive papilla and first palatal ridge
area between second palatal ridge and first upper molar
area between first upper molar and nasopharynx.

Result : All parental animals survived to scheduled sacrifice and no treatment related clinical signs were observed.
Except for a slight reduction in body weights in the high dose males there were no other effects on either body weight or food consumption. When compared to the controls, at week

3 the decrease in weight of the high dose males was 3.8% and at week 7 was 7.8.
The only treatment related organ weight changes were an increase in relative kidney (15%) and relative liver (5%) weights in the high dose males. No other organ weight changes were recorded.
There were no treatment-related microscopic changes in the testes, epididymes, ovaries or nasal turbinates in the animals in the high dose group.

Reproductive/fertility effects

All groups had a mating index and a fertility index of 100% and all animals in all groups had mated within 4 days of cohabitation.
Delivery and litter data did not demonstrate any effects of treatment see data summarized below.

Parameter	Dose group (ppm)			
	0	750	2500	7500
Females on study	10	10	10	10
Litters with liveborn	10	10	10	10
Implantation sites	147	154	155	154
Mean	14.7	15.1	15.5	15.4
Pups delivered (total)	145	151	146	145
Liveborn	142	151	143	144
Live birth index (%)	98	100	98	99
Pups dying				
Day 0	0	1	1	1
Days 1-4	2	4	0	0
Pups surviving 4 days	140	146	142	143
Viability index (%)	99	97	99	99
pup sex distribution				
Day 0 M/F (ratio)	63/79	67/84	69/74	68/76
Day 4 M/F (ratio)	63/77	64/82	68/74	68/75
Pup weight/litter (g)				
Day 0	6.0	6.6	6.2	6.1
Day 4	9.3	8.9	9.2	9.6

External and internal examination of pups sacrificed on day 4 of lactation resulted in only one pup in a single litter of the control group with abnormalities.

Reliability : (1) valid without restriction
19.11.2001 (23)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	Gestation days 6-19 inc.
Frequency of treatment	:	6 hours/ day
Doses	:	Target concentrations: 2000 and 8000 ppm. Actual: 2160 and 7800 ppm
Control group	:	yes
NOAEL Maternalt.	:	7800 ppm
NOAEL Teratogen	:	7800 ppm
Year	:	1996
GLP	:	no data
Test substance	:	Partially vaporized full range catalytic reformed naphtha
Method	:	Groups of 11 or 12 presumed pregnant female rats were exposed 6 hours each day from days 6-19 of gestation to whole body exposures of 2000 or 8000 ppm partially vaporized FRCRN. Two extra groups served as untreated and sham treated controls. All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation. On day 20 each female was sacrificed and blood samples removed for serum chemistry evaluations. Parameters measured were the same as those in the subchronic study by the same authors, and in addition included iron and lactic dehydrogenase. All organs were examined grossly and liver and thymus weights were recorded. In addition, the number of corporea lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early and late resorptions and live and dead fetuses were recorded. Each fetus was gendered, weighed and grossly examined for external abnormalities. Half the fetuses were fixed in Bouin's fluid and examined subsequently for soft tissue abnormalities. Remaining fetuses were stained with Alizarin red and examined for skeletal anomalies.
Result	:	There were no adverse effects on maternal body weight gain, liver weight or thymus weight. In the high dose group, maternal serum glucose levels were significantly decreased (1.5%) and potassium levels increased (1%) relative to the untreated controls. Reproductive performance during gestation and in-utero survival and development of concepti were unaffected by treatment. Furthermore, there were no treatment-related increases in gross abnormalities or anomalies of soft or skeletal tissues.
Reliability	:	(1) valid without restriction

19.11.2001 (14)

(1) ABC Laboratories, Inc (1998)
Method Validation for the Analysis of the Water Accomodated Fraction of Light Catalytically Cracked Naphtha using Purge-and-Trap and GC/FID, Study No. 43582.
ABC Laboratories, Inc. Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri

(2) ABC Laboratories, Inc. (1998)
Static Renewal 96 Hour Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Catalytically Reformed Naphtha, LCRN to Fathead Minnow (Pimephales promelas). Project ID. 43578.
Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri.

(3) ABC Laboratories, Inc. (1998)
Static Renewal 48-hour Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Catalytically Reformed Naphtha, LCRN) to Daphnia Magna. Study No. 43577.
Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri. 1998.

(4) ABC Laboratories, Inc. (1998).
Static Renewal 96 Hour Acute Toxicity of the Water Accomodated Fraction (WAF) of Light Catalytically Reformed Naphtha, LCRN to to a Freshwater Alga, Selenastrum capricornutum. Project ID. 43579.
ABC Laboratories, Inc. Environmental Toxicology, 7200 E. ABC Lane, Columbia, Missouri.

(5) American Petroleum Institute (1984)
Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats, full range catalytically reformed naphtha, API sample 83-05.
Study conducted by Litton Bionetics, Inc.
API Medical Research Publication No. 31-30681, February 1984.

(6) American Petroleum Institute (1985)
Acute oral toxicity study in rats, acute dermal toxicity study in rabbits, primary dermal irritation study in rabbits, primary eye irritation study in rabbits of API 83-05 full range catalytically reformed naphtha.
Study conducted by Hazleton Laboratories America, Inc.
API Med research publication N0.32-31474, April 1985.

(7) American Petroleum Institute (1985)
Mutagenicity evaluation of 83-05 in the rat bone marrow cytogentic assay.
Study conducted by Litton Bionetics, Inc.
API Mes. Res. Publ. 32-32289, June 1985.

(8) American Petroleum Institute (1985)
Mutagenicity evaluation of catalytically reformed naphtha API #83-05 in the mouse lymphoma forward mutation assay.
Study conducted by Litton Bionetics, Inc.
API Med. Res. Publ. 32-32459, July 1985.

(9) American Petroleum Institute (1986)
28-day dermal toxicity study in the rabbit, API 83-05, full range catalytically reformed naphtha (CAS 68955-35-1)
Study conducted by Tegeris Laboratories Inc.
API HESD Research Publication 33-30598, February 1986.

(10) American Petroleum Institute (1986)
Dermal sensitization study in guinea pigs, API 83-05, full range catalytically reformed naphtha.
Study conducted by Hazleton Laboratories America Inc.
API HESD Research Publication 33-30497, Januaary 1986.

(11) CONCAWE (1992)
Ecotoxicological Testing Of Petroleum Products : Test Methodology.
Report 92/56,
CONCAWE, Brussels.

(12) CONCAWE (1995)
Algal, Growth Inhibition Test: study no. 104767, test substance MRD-95-047.
Study conducted by Exxon Biomedical Sciences Inc.

(13) CONCAWE (1996)
Environmental risk assessment of petroleum substances: the hydrocarbon block method.
Report 96/52,
CONCAWE, Brussels.

(14) Dalbey, W. and Feuston, M. (1996)
Partially vaporized full range catalytic reformed naphtha: Subchronic and developmental toxicity studies in rats.
Inhalation Toxicology. Vol 8., pp 271-284

(15) ECETOC (1998)
QSARS in the Assessment of the Environmental Fate and Effects of Chemicals. Technical Report No. 74

(16) ECETOC, (1996)
Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances. Monograph 26.

(17) Harris, J.C. (1982)
Rate of Hydrolysis. In Handbook of Chemical Property Estimation Methods. p. 7-6. W. J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY, USA.

(18) Mackay, D, A. DiGuardo, S. Paterson, & C. Cowan (1997)
EQC Model, ver. 1.01, 1997, available from the Environmental Modelling Centre, Trent University, Canada.

(19) Meylan, M, SRC 1994-1999.
LOGKOWWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(20) Meylan, M, SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN (Estimate ver. 3.04), available from Syracuse Research Corp.

(21) Peterson, D.R., (1994)
Calculating the aquatic toxicity of hydrocarbon mixtures
Chemosphere Vol.29, 12, pp. 2493-2506

(22) Schreiner, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Lapadula, E., Podhasky, P., White, R., Hoffman, G. and Mandella, R. (2000)
Toxicity evaluation of petroleum blending streams: Inhalation subchronic toxicity/neurotoxicity study of a light catalytic reformed naphtha distillate in rats.
J. Tox. and Env. Health, Part A. Vol. 60, pp 489-512

(23) Schreiner, C., Bui, Q., Brelia, R., Burnett, D., Koschier, F., Podhasky, P., White, R., Hoffman, G. and Schroder, R. (2000)
Toxicity evaluation of petroleum blending streams: reproductive and developmental effects of light catalytic reformed naphtha distillate in rats.
J. Tox. and Env. Health, part A., Vol 60, pp 101-116

(24) Springborn Laboratories, Inc. (1999)
Light Catalytically Reformed Naphtha-Determination of Inherent Biodegradability. Study No. 13687.6110